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CONTENTS

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BRACHIOPODEN IN DER ONAGAWA-BUCHT AM SÜDENDE DES KITAKAMI-GEBIRGES NORDÖSTLICH VON SENDAI, NORD-JAPAN

VON

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(Mit drei Text-Abbildungen)

(Eingegangen am 1. März 1938)

Die mir von Herrn T. IMAI vom Ozeanochemischen Institut der kaiser[l] Tohoku Universität zu Onagawa, zum Studium anvertraute, klein Brachiopodenfauna, die in der Umgebung von Onagawa (Text-Abbildunge[n] 1 und 2) gesammelt wurde, besteht aus den folgenden Formen.

Lingula cf. *nipponica* HAYASAKA

Lingula spp. indet.

Hemithyris psittacea (GMELIN) subsp. *woodwardi* ADAMS

Terebratalia coreanica (ADAMS et REEVE)

Terbratulina kitakamiensis, sp. nov.

Coptothyris grayi (DAVIDSON) subsp. *aomoriensis* HAYASAKA

Diese Fauna enthält eine neue Art, *Terebratulina kitakamiensis*, abe es ist keineswegs eine neue Entdeckung; nur ist es in dieser Gelegenhei spezifisch benannt. *Lingula* ist in dieser Fauna nur sehr spärlich vertreten. Die übrigen sind die Arten, die uns schon lange mehr oder weniger familiär gewesen sind; nach unserer gegenwärtigen Kenntnis sind sie fast ausschliesslich nordjapanisch in Verbreitung.

Die Formenarmut der ausschliesslich nordjapanischen Brachiopoden wird immer deutlicher, dennoch scheinen einerseits diese wenigen Arten ziemlich weit verbreitet und anderseits manchmal sehr reich an Individuenzahl zu sein.

Vor der Beschreibung der Arten bezeige ich Herrn IMAI meine Erkenntlichkeit dafür dass er mir das Material zur Untersuchung angeboten hat.
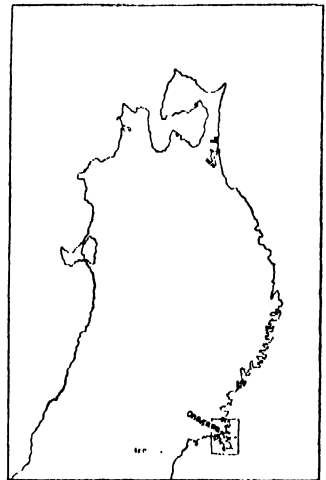
Text-Abbildung 1. Die Lage der Onagawa-Bucht.

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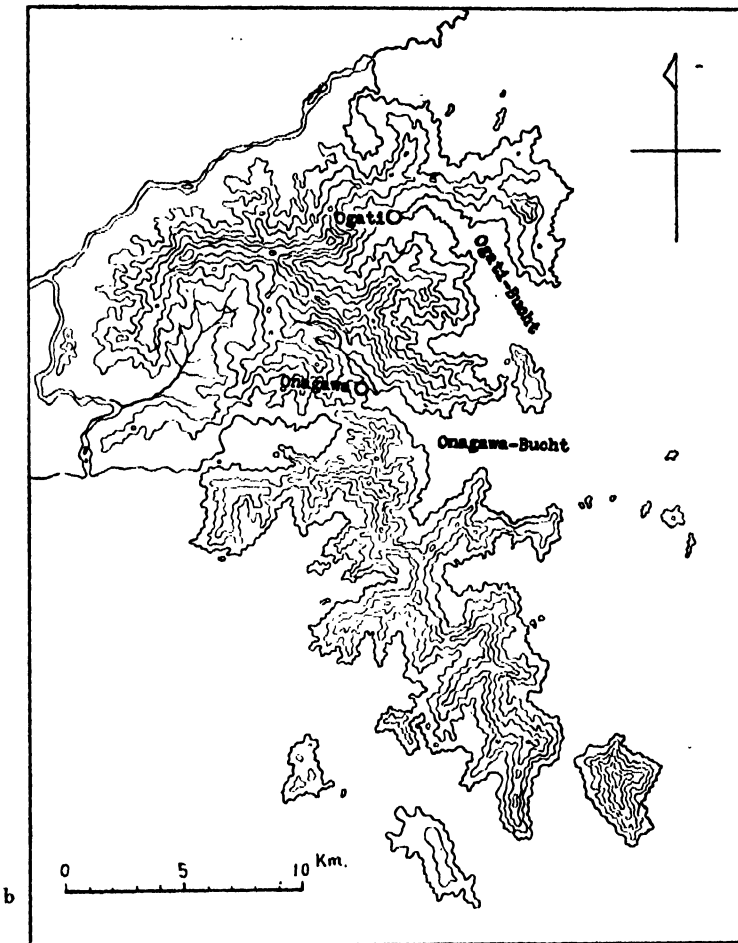
Text-Abbildung 2. Die Umgebung von der Onagawa-Bucht.

Lingula cf. nipponica HAYASAKA

1932. *Lingula nipponica*, HAYASAKA: — Sci. Rep. Tôhôku Imp. Univ. 4th ser. (Biology), vol.
VII, No. 1, p. 2, pl. I, fig. 1; pl. II, fig. 1.

Eine kleine Klappe, deren Schlossrand abgebrochen ist. Die Länge
dieser unvollständigen Schale misst 15 mm, während sie etwa 6.5 mm.
breit ist; der Umriss stimmt mit dem der *Lingula nipponica* fast überein,
obwohl der Stirnrand etwas mehr gerundet ist.

Die Fundstelle: — 699. Hier wurde diese Schale mit einem Fragmente
einer grösseren, unbestimmbaren Spezies gefunden, die sich entweder von

L. nipponica oder von der hier beschriebenen verwandten Form entschieden unterscheidet.

Der Fundstelle 573 stammte noch eine andere fragmentarische *Lingula-Schale* ab, die mit einem Stückchen der Schale der *Hemithyris* gesammelt wurde.

Hemithyris psittacea (GMELIN) subsp. woodwardi ADAMS

1863. *Rhynchonella woodwardi*, ADAMS: — Ann. Mag. Nat. Hist., 3rd ser. vol. XI, p. 100.

1886. *Rh. psittacea* var. *woodwardi*, DAVIDSON: — Trans. Lim. Soc. London, 2nd ser. (Zoology), vol. IV, p. 168, pl. XXIV. figs. 12, 13.

1932. *Hemithyris psittacea* subsp. *woodwardi*, HAYASAKA: — Sci. Rep. Tôhôku Imp. Univ. 4th ser. (Biology), vol. VII, No. 1, p. 4, pl. I, fig. 2; pl. II, fig. 2.

Das Vorkommen der zwei sehr kleinen nepionischen Exemplare ausgenommen, gibt es nichts besonderes zu erwähnen. Sie stammten der Fundstelle 912 ab. Das grössere ist nicht grösser als 3.6 mm in Länge, 3.4 mm in Breite und 1.1 mm in Höhe; das andere ist nur 2.3 mm lang. Was die Schalenform betrifft, ähneln sich diese beiden winzigen Individuen der vor kurzem von YABE und HATAI beschriebenen neuen Art *H. pellucida*[1], dennoch ist der Stirnrand nicht gerade wie bei der letzten, sondern schwach abwärts umgebogen, d. i., „sulcate" nach THOMSON[2].

Die Fundstellen: — 910. Zwei erwachsene Exemplare, 910 (l) und 910 (p). An 912 fanden sich die winzigen nepionischen Schalen.

Für das Weitere über diese Form empfiehlt sich meine oben zitierte Schrift, s. 4–6.

Terebratalia coreanica (ADAMS et REEVE)

1882 *Terebratella coreanica*, DUNKER: — Index Molluscorum Maris Japonici, p. 252.

1887. —— ——, DAVIDSON: — Trans. Linn. Soc. London, 2nd ser. (Zoology), vol. IV, p. 81, pl. XXXIII, figs. 4–7.

1920. *Terebratalia coreanica*, DALL: — Proc. U. S. Nat. Mus., vol. 57, p. 345.

1932. —— ——, HAYASAKA: — Sci. Rep. Tôhôku Imp. Univ., 4th ser. (Biology), vol. VII, No. 1, p. 7, pl. I, fig. 4; pl. II, figs. 4–7.

1934. —— ——, NOMURA and HATAI: — Saito Ho-on Kai Museum Bull. No. 2, p. 10, pl. II, figs. 1–6.

1935. —— ——, NOMURA and HATAI: — Saito Ho-on Kai Museum Bull. No. 6, p. 239.

Die mir anvertraute Sammlung enthält 19 Exemplare dieser schönen Art, die hauptsächlich innerhalb der Bucht von Onagawa gesammelt wurden. Die paar jungen Individuen haben quer ovalen Umriss und sind fast immer bilateral-symmetrisch, während die erwachsenen, grösseren

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[1] Proc. Imp. Academy X, No. 10, p. 661, figs. 1–4, 1934.

[2] Brachiopod Morphology and Genera, s. 58, 1927.

Exemplare, die länglich oval im Umrisse sind, eine deutliche Neigung zur Asymmetrie zeigen. Denjenigen, denen diese populäre Art nicht wohl bekannt ist, wird eine der oben erwähnten Schriften von NOMURA und HATAI (1935)[1] empfohlen, um sich den Begriff der Eigentümlichkeiten der Art sowie der Veränderlichkeit ihrer Schalenform daraus verschaffen zu können. Hier werden nur die Dimensionen einiger Exemplare gegeben, um die Abhängigkeit der Schalengrösse von der Symmetrie resp. Asymmetrie des Schalenumrisses anzudeuten. Die Abweichung der Schalenform der von mir behandelten Exemplare überhaupt von der von DAVIDSON in seiner Abhandlung (1887) beschriebenen ist ganz gleich mit derjenigen, die ich schon in Bezug auf die Formen der Mutsu-Bai wahrgenommen habe[2].

Nr. d. Fundstelle	Schalenlänge	Schalenbreite	Schalenhöhe
708 (c)	56 mm.	49.5 mm.	35 mm.
915 (a)	56	53.5	35
596 (a)	45	43.5	34
557 (l)	48	43	33
1076	45	42	28.5
,,	42	48	26
450	39	42	26 (bilateral symmetrisch)
,,	39	38	28 (,,)
1076	30	33	28 (,,)
450	21	24	11 (,,)
,,	21	22	10 (,,)

Fundstellen: ~450, 596, 639, 686, 708, 915, 1076 (Innerhalb der Onagawa-Bucht); 557 (in der kleinen Bucht von Ogati nördlich von Onagawa).

Verbreitung: — *Terebratalia coreanica* wird ausschliesslich in Nord-Japan gefunden, die bisher bekannte südlichste Grenze scheint aber die See um die Halbinsel Bôsô südöstlich von Tôkyô zu sein; Fossilien[3] finden sich

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[1] A Note on Two Species of Brachiopoda: Pt. I. On the Variation, Geologic and Geographic Distribution, Geologic Significance, Systematic Position, and Evolutionary Trends of *Terebratalia coreanica* (ADAMS and REEVE).

[2] Brachiopods of Mutsu Bay, p. 7.

[3] Das fossile Vorkommen dieser Art wurde etwa in den folgenden Schriften behandelt.

M. YOKOYAMA: — Jour. Coll. Sci. Imp. Univ. Tôkyô, vol. XXXIX, Art. 6, p. 184, pl. XIX, figs. 25, 28, (1920): *Ibid*, vol. XLIV, Art. 1, p. 198 (1922): *Ibid*, vol, XLIV, Art. 7, p. 8 (1923): Jour. Facult. Sci. Imp. Univ. Tôkyô, Sec. II, vol. I, pt. 8, p. 309 (1926)

I. HAYASAKA: — Sci. Rep. Tôhoku Imp. Univ. 2nd ser. (Geology), vol. VI, No. 2, p. 149, 160, pl. VII, fig. 27; pl. VIII, fig. 1 (1922).

S. NOMURA and K. HATAI: — Saito Ho-on Kai Museum Research Bull. No. 5, p. 57 (1935).

K. HATAI: — Jap. Jour. Geol. and Geogr. vol. XIII, Nos. 3/4, p. 309 (1936).

etwas weiter in der südwestlichen Gegend; geologisch wird das Alter des
Fossils dieser Spezies am ältesten als Miozän angegeben.

Terebratulina kitakamiensis, sp. nov.

(Text-Abbildung 3)

1932. *Terebratulina* sp. indet., HAYASAKA: — Sci. Rep. Tôhoku Imp. Univ. 4th ser. (Biology),
vol. VII, No. 1, p. 6, pl. I, fig. 3.

Nur zwei Exemplare: das eine (891 g') ist nur durch eine Dorsalschale
vertreten, das andere (910 m) dagegen ist mit dem charakteristischen
Armgerüst drinnen versehen. Dessen Schalenlänge misst 17 mm, die
Schalenbreite 11 mm und die Schalenhöhe 9 mm. Die mehr oder minder
unregelmässige Schalenform sowie der unsymmetrische Umriss sollen als
diesem Individuum eigentümlich betrachtet werden.

Dieselben Charakteristika wurden bei dem Exemplare beobachtet, das
ich als eine unbestimmbare Art der Gattung von Mutsu-Bai beschrieben
habe. Überdies ist alles, was über das letzte bemerkt wurde, bei dem
hier behandelten Exemplare in gleicher Weise gültig; nämlich, (a) die
verhältnismässig groben Längsstreifen; (b) einige, mehr oder minder
deutliche, konzentrische Anwachswurzeln; (c) der schwach abgeplattete
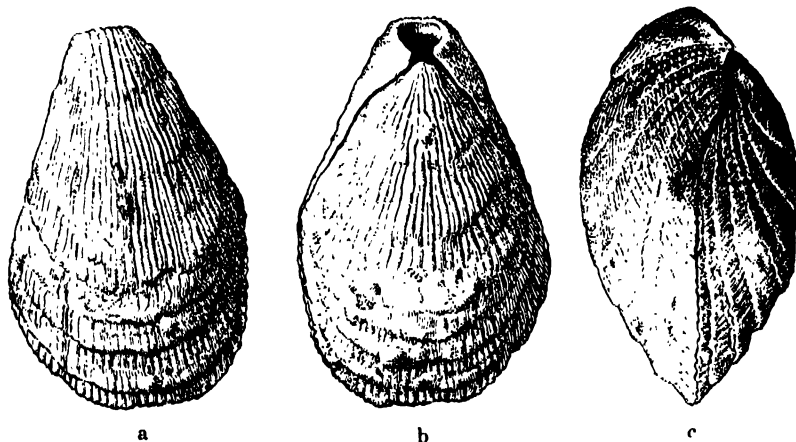
Text-Abbildung 3. *Terebratulina kitakamiensis* HAYASAKA. (a) Ventrale,
(b) dorsale und (c) laterale Ansichten (ca.×3).

Vorderrand der Ventralschale. Das letzte Merkmal scheint die Tendenz
zur Entwicklung der Mediandepression anzudeuten, also dadurch nähert
sich diese Spezies der kurz vorher von HATAI und NOMURA beschriebenen

Form *Terebratula japonica*[1] (non SOWERBY). Bezüglich der Schalengrösse ist diese Form viel kleiner als die von DAVIDSON als normal betrachteten Exemplare; das kleinste (Taf. III, fig. 11 von DAVIDSON) nähert sich dem meinigen, aber es zeigt die asymmetrische und unregelmässige Schalenform nicht. Aus diesen Beziehungen könnte *T. kitakamiensis* die Stelle der weiter im Süden verbreiteten *T. japonica* im nordjapanischen Meers einnehmen. Der Artname stammt vom Kitakami-Gebirgsland, da diese Art in der See um dieses Gebirge gefunden wurde.

Die Fundstellen sind, wie schon erwähnt, 891 und 910.

Verbreitung: — Mutsu-Bai, Aomori-Ken und Onagawa-Bucht, Miyagi-Ken.

Coptothyris grayi (DAVIDSON) subsp. aomoriensis HAYASAKA

1932. *Coptothyris grayi* subsp. *aomoriensis*, HAYASAKA:— Sci. Rep. Tôhoku Imp. Univ. 4th ser. (Biology), vol. VII, No. 1, p. 9, pl. 1, figs. 5, 6.

1933. *Coptothyris grayi aomoriensis* fossilis, HAYASAKA:— Japanese Journal of Geology and Geography, vol. X, Nos. 3/4, p. 125, pl. XII, figs. 1-6.

1934. *Coptothyris grayi*, NOMURA and HATAI:— Saito Ho-on Kai Museum Research Bull. No. 2, p. 13, pl. 1, figs. 1-6; (?) figs. 7, 8.

Die Schalen von der Onagawa-Bucht sind überhaupt kleiner als diejenigen von Mutsu-Bai, die von mir wie von NOMURA und HATAI beschrieben und abgebildet wurden. Die Schalenform und die Entwicklungsweise der Radialrippen entsprechen der Beschreibung der Exemplare von Mutsu-Bai, die 1932 veröffentlicht wurde. Einige transversale Schalen aber scheinen sich der typischen Form *C. grayi* betreffs des Schalenumrisses mehr oder weniger zu nähern. Bezüglich der Schalenskulptur jedoch weichen sie von einander deutlich ab.

Bemerkenswert ist die Tatsache, dass die sehr kleinen nepionischen Individuen sich mehr der Spezies *C. grayi* als der Varietät *aomoriensis* ähneln, indem sie ausschliesslich breiter als lang sind, ihre Berippung einfacher ist und die Rippen viel weniger sind. Diese kleinen Schalen stammten der Fundstelle 648 ab, während die grösseren, erwachsenen Formen an der Stelle 657 gesammelt wurden. Das grösste Exemplar von den letzten misst: die Länge 32.5 mm, die Breite 31.5 mm, die Höhe 18.5 mm. Die nepionischen Formen sind etwa 4 mm : 5.2 mm in Länge und Breite.

Nach meiner Ansicht ist *C. |grayi aomoriensis* der nordjapanische Vertreter der im Süden ziemlich weit verbreiteten Form *C. grayi* betrachtet

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[1] Saitô Ho-on Kai Museum Research Bull. No. 2, p. s. 8, Taf. II, fig. 25-30, 1934.

zu werden: die beiden scheinen überhaupt in ihrem nepionischen Entwicklungsstadium identisch zu sein.

Die Fundstellen: — 657 (viele erwachsene Formen); 648 (3 nepionische Schalen).

Die Verbreitung: — Ostküste Nordjapans.

FERTILIZATION AND EARLY EMBRYOGENY OF CHAMAECYPARIS PISIFERA S. Z.

BY

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We have at present only a few papers bearing on the gametophyte, fertilization and embryogeny of the genus *Chamaecyparis*. COKER (1904) described the two-celled pollen grains of some species of this genus, LAWSON (1907) states in his report on the Cupressineae that he obtained a complete series of the fertilization of *Chamaecyparis* sp. but he has not illustrated it. In 1932 BUCHHOLZ reported on the embryogeny of *Ch. obtusa*. With regard to the chromosome number, SAX and SAX (1933) reported that it is eleven in the prothallial cells of *Ch. Lawsoniana*.

The material used in this work was obtained from a large tree in the grounds of a shrine, Kameoka-Hachiman, in Sendai. The fixation was made by a chrom-acetic strong solution, after dipping the material into CARNOY's triple solution for a few minutes. For staining, NEWTON's gentian violet iodine or FEULGEN's nuclear staining was used.

THE MALE AND FEMALE GAMETOPHYTE

In *Chamaecyparis pisifera*, archegonia are grouped together in a single complex at the apex of the prothallium. They vary in number from five to twelve, seven being most common. On the outside of the complex a single layer of the jacket cells is found. The neck cells vary in number from eight to twelve which are disposed in one or two tiers. The ventral canal nucleus soon disorganizes after its formation from the central nucleus.

Two male gametes of equal size are formed by a division of the body cell and are found just behind the stalk- and tube-nuclei at the tip of the pollen tube. The male nuclei have their own cytoplasm, sharply limited from the general cytoplasm of the pollen-tube, as in other species of the Cupressineae.

FERTILIZATION AND EARLY EMBRYOGENY

In 1937 the fertilization took place about the 20th of June. In an archegonial complex, generally several archegonia are fertilized at one time. The writer found a case, for example, in which eight archegonia of a complex consisting of nine archegonia were in the stage of the fertilization.

After the entrance of the male gamete into the archegonium the male nucleus escapes from its surrounding cytoplasm and advances towards the egg nucleus. The male and female nuclei contact at the upper part, more or less, of the middle portion of the archegonium (Pl. I, Fig. 1) and both the nuclei in this condition pass down to the bottom of the archegonium (Fig. 2). This statement is totally different from LAWSON's description (1907).

In the course of sinking towards the archegonial bottom, the relative position of the male and female nuclei often changes, the male nucleus being found sometimes situated under the female nucleus.

The long axis of the first proembryonal division varies in its position, being sometimes parallel and sometimes perpendicular to the long axis of the archegonium (Fig. 3). As a result of this division two free daughter nuclei are formed. Figs. 4 and 5 show the stages of the third division. After this division the cell walls are formed, so that the free nuclear condition ceases at the stage of four free nuclei (Figs. 6 and 7). As a result of the wall formation, two tiers of cells are formed, generally five or six above, and two or three below (Fig. 7) and the upper tier is always open above. In the next stadium the upper tier divides into two layers (Fig. 8), so three tiers of cells are formed, the uppermost tier also remaining open to the general cytoplasm of the archegonium. In the later stage the second tier becomes the prosuspensor (Fig. 9) and the lowest tier forms the embryo proper, the embryonal-tube and the primary suspensor. The division of the lowest tier always takes place before the elongation of the second tier. The results of the writer's observation concerning the further development of the embryo coincide with BUCHHOLZ's account on *Ch. obtusa* (1932).

One or more supernumerary nuclei are sometimes found at the upper part of the archegonium (Text-fig. 1). The origin of these nuclei is not clear, but it seems probable that they are stalk- and tube-nuclei which have entered into an archegonium at the fertilization; in some exceptional cases numerous nuclei of different sizes appear in the same region. The

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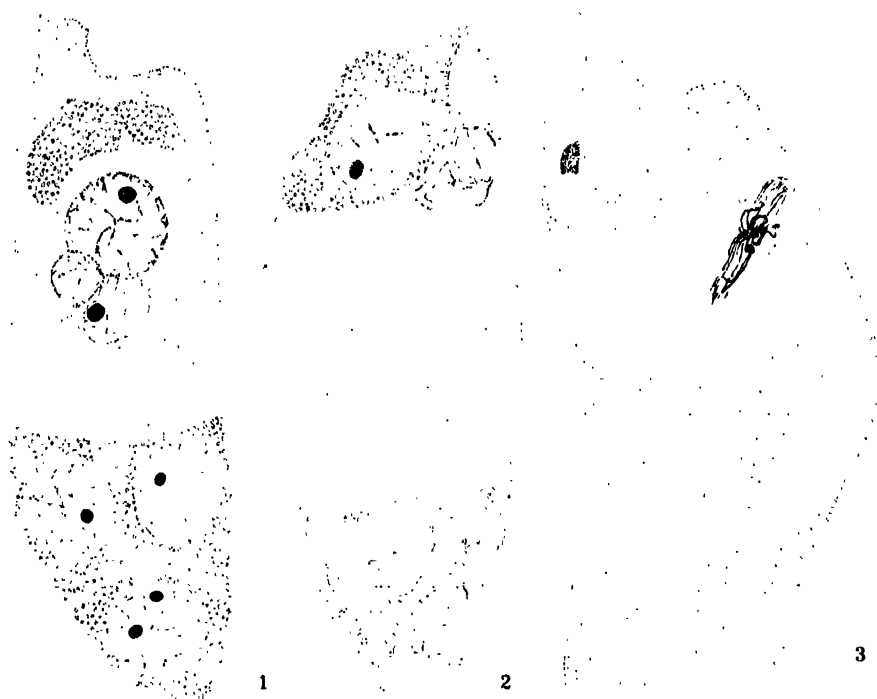
Text-figs. 1. Extra nuclei in an archegonium with a proembryo. 2. Second male gamete in an archegonium with a proembryo. Beside the male gamete another questionable nucleus is seen. 3. Division of the extra nucleus. × 560

mitotic figures of these additional nuclei were also found, being, however, more of less irregular (Text-fig. 3). In one case the entrance of a second male nucleus with its own cytoplasm into a normal archegonium was observed (Text fig. 2).

NUCLEI IN THE JACKET CELLS

The haploid chromosome number of this plant was estimated to be eleven in the divisions of the central nucleus (Text-figs. 4, 5) and in the jacket cells (Text-fig. 6). This number coincides with that of *Ch. Lawsoniana* determined by SAX (1933). One of eleven chromosomes of *Ch. pisifera* is distinguished by the possession of a large trabant (Text-figs. 6, 7), so that at the telophase one nucleolus appears in each daughter nucleus (Text-fig. 8).

It is remarkable that after the fertilization some jacket cells have
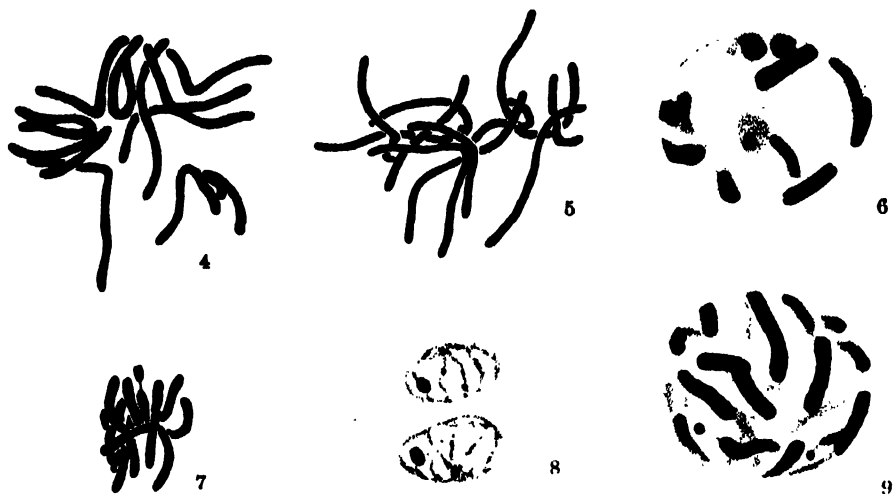
Text-figs. 4 & 5. Division of the central nucleus of the archegonium. 4. Polar view. 5. Side view. 6. Prophase of a jacket nucleus; one chromosome has a trabant and is seen connected with a nucleolus. 7. Side view of a female prothalial mitosis. One chromosome has a large trabant. 8. Telophase of a female prothallial mitosis. Daughter nuclei have each only one nucleolus. 9. Prophase of a diploid jacket nucleus; two chromosomes have each a large trabant. ×1790
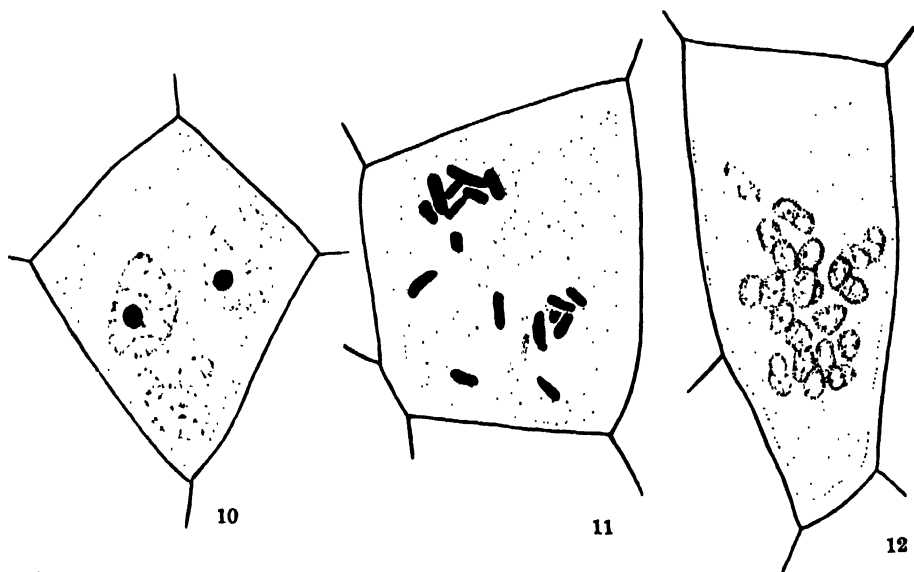
Text-figs. 10. Caryomeres of a jacket cell. 11. Prophase of the caryomeres in a jacket cell. 12. About twenty-two caryomeres in a jacket cell. ×1790

nuclei showing the diploid number of chromosomes (Text-fig. 9) and other jacket cells have a number of nuclei of different sizes (Text-figs. 10, 12). Text-fig. 11 shows the prophase of the mitotic divisions of these numerous nuclei; different numbers of chromosomes are found in each nucleus. In some cases about twenty-two small nuclei are counted in a cell (Text-fig. 12), they are perhaps the caryomeres formed at the telophase of a mitotic division of a haploid nucleus.

SUMMARY

The male and female gametophytes, and the fertilization and early embryogeny of *Ch. pisifera* S. Z. are described. The chromosome number of this species is eleven in the haploid generation. One among these eleven chromosomes has a large trabant. Later in jacket celles, nuclei showing the diploid number of chromosomes or caryomeres were observed.

LITERATURE

1) BUCHHOLZ, J. T. 1932. The embryogeny of *Chamaecyparis obtusa.* Amer. Jour. Bot. **19**, 230–238. 7 text-figs.
2) COKER, W. C. 1904. On the spores of the Coniferae. Bot. Gaz. **38**, 206–213. 24 text-figs.
3) LAWSON, A. A. 1907. The gametophytes and embryo of Cupressineae, with special reference to *Libocedrus decurrens.* Ann. Bot. **21**, 281–303. pls. 24–26.
4) SAX, K. & H. J. SAX. 1933. Chromosome number and morphology in the conifers. Jour. Arnold Arb. **14**, 356–375. pls. 75–79, 3 text-figs.
5) SCHNARF, K. 1933. Embryologie der Gymnospermen. Berlin.

EXPLANATION OF THE PLATE

(Magnification ×650.)

1. Contact of the male and female nuclei at the fertilization.
2. Contacted nuclei at the bottom of an archegonium.
3. Proembryonal first division at the bottom of an archegonium
4 & 5. Proembryonal third division in two successive sections.
6. Eight nuclei formed by the third division.
7. Eight nucleus stage after the wall formation.
8. Mitosis in the upper tier.
9. Early stage of the prosuspensor elongation. The uppermost "Offenzellen" show a tendency toward degeneration.

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Y. SUGIHARA: Fertilization and Early Embryogeny of Chamaecyparis
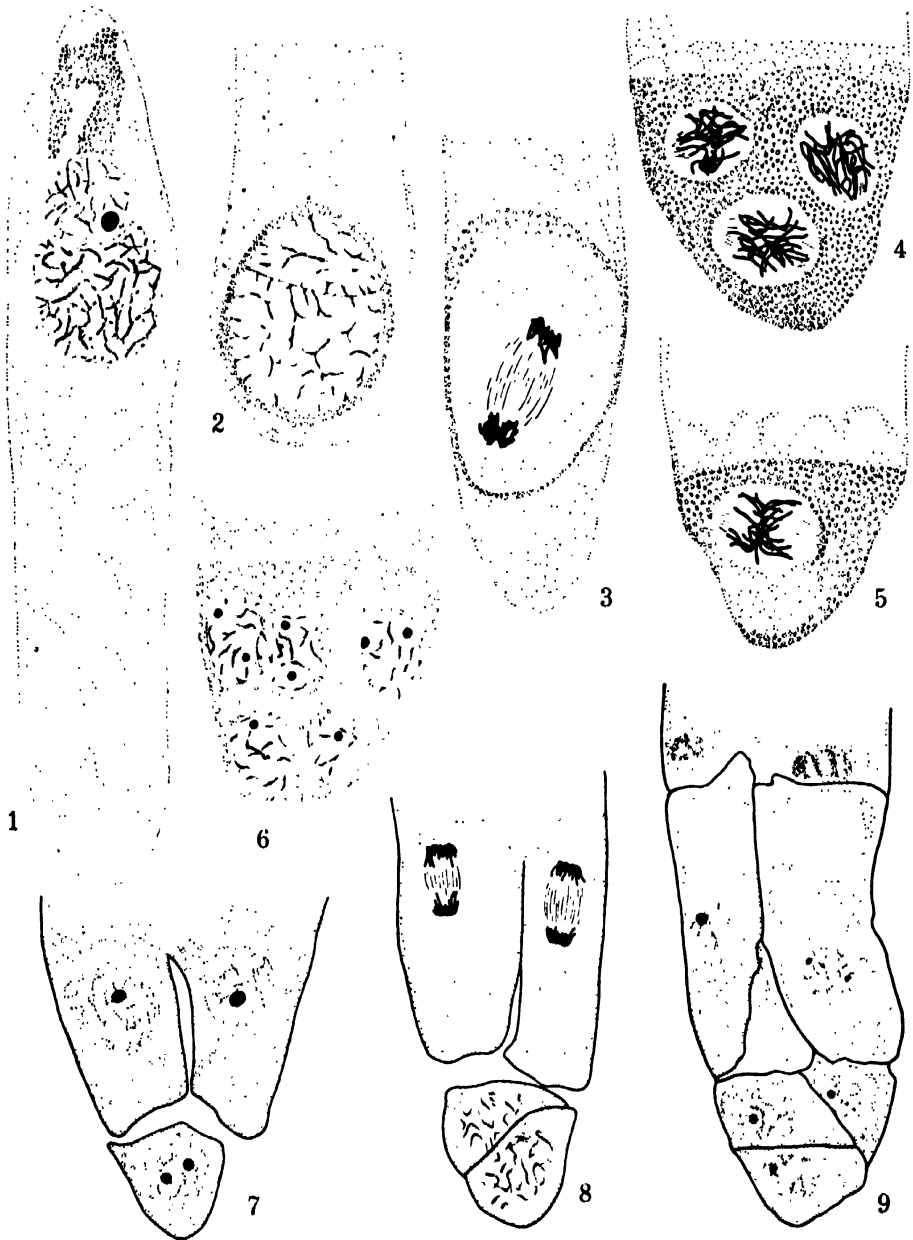
SYSTEMATIC RELATIVE GROWTH IN CYPRAEA-ERRONEA-PUSTULARIA-TALPARIA GROUP

BY

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(With one Figure)

(Received March 29, 1938)

Morphologically similar species are collected and classified in a genus and in a family by taxonomists. These species are recognizable as those descending from an ancestral type. Among them, therefore, in so far as the evolutionary theory is assumed, there ought to exist some definite, continuous relations both in their quantitative and qualitative characteristics. Hitherto, taxonomists have implied this fact but in a few vague statements merely. In this paper, an attempt has been made to define some quantitative relations in morphological characteristics with the notion of relative growth. Since the number of observations is very small, greater validity may only be expected as the result of more numerous observations; nevertheless the facts obtained by this observation are extremely clear. The existence of these relations has been established in regard to the genera, *Cypraea*, *Erronea*, *Pustularia* and *Talparia*, and, further, to the group which includes these genera as a whole.

The writer is indebted to Prof. E. NOMURA for his kind guidance, to Mr. H. SHIMATZ for the advantage of having been able to use the measurements of specimens in his collection, and also to Mr. T. KURODA for the information regarding the specific names, *viz. Erronea marmorata* (SCHRÖTER), *E. melvilli* (HIDALGO), *E. hirundo* (L.), *E. artuffeli* (JOUSSEAUME), *Pustularia magistra differens* SCHILDER, *P. boivinii* (KIENER), and *Trivirostra oryza* (LAMARCK). The classification is based on HIRASE's monograph[1].

SYSTEMATIC RELATIVE GROWTH

It has been observed in the case of many animals that a species grows according to the law of allometry ($y = bx^a$), *i. e.* as regards two dimensions, *e. g.* the breabth and length, or the height and length. In the case of

[1] HIRASE, S. 1934. A Collection of Japanese Shells with Illustrations in Natural Colours. Tôkyô.

TABLE 1.

Species	No.	Mean length (cm)	Mean breadth (cm)	Mean height (cm)
Cypraea carneola L.	3	2.74	1.63	1.36
C. vanelli L.	4	4.13	2.35	1.96
C. vitellus L.	3	4.84	2.96	2.48
C. arabica L.	3	5.82	3.69	2.93
C. tigris L.	1	7.20	4.96	3.85
Erronea marmorata (SCHRÖTER)	8	1.13	0.64	0.53
E. punctata atomaria (GMELIN)	1	1.30.	0.75	0.62
E. ziczac (L.)	2	1.67	0.98	0.81
E. hirunda (L.)	2	1.68	1.04	0.83
E. japonica SCHILDER	29	1.70	1.01	0.82
E. artuffeli (JOUSSEAUME)	19	1.70	1.01	0.83
E. melvilli (HIDALGO)	4	1.74	0.97	0.75
E. asellus (L.)	4	2.00	1.22	0.96
E. cribraria (L.)	1	2.06	1.16	0.95
E. errones (L.)	4	2.72	1.50	1.27
E. cylindrica BORN	2	2.91	1.61	1.11
E. chinensis (GMELIN)	2	3.34	1.99	1.64
E. onyx (L.)	2	3.84	2.32	1.89
Pustularia cicercula (L.)	4	1.45	0.86	0.79
P. poraria (L.)	1	1.81	1.23	0.95
P. childreni (GRAY)	1	1.88	1.35	1.07
P. moneta (L.)	8	2.01	1.46	0.96
P. labrolineata (GASKONI)	8	2.04	1.24	1.04
P. limacina (LAMARCK)	1	2.09	1.21	0.95
P. helvola (L.)	4	2.34	1.56	1.17
P. annulus (L.)	6	2.41	1.74	1.19
P. nucleus (L.)	2	2.45	1.55	1.19
P. cernica (SOWERBY)	1	2.46	1.70	1.32
P. staphylaea (L.)	5	2.56	1.50	1.23
P. boivinii (KIENER)	7	3.09	1.90	1.48
P. caputserpentis (L.)	8	3.16	2.21	1.59
P. erosa (L.)	2	3.31	2.07	1.49
P. magistra differens SCHILDER	2	3.40	2.12	1.73
Talparia isabella (L.)	2	2.57	1.41	1.20
Trivirostra oryza (LAMARCK)	13	0.69	0.50	0.43

Erronea japonica and *E. artuffeli*, this relation has also been assumed, and, further it may be fairly presumed regarding the other species which

are allied to the two here mentioned. The allometrical equation has, therefore, been applied to some of these species, the number of specimens of which is comparatively large. Further, it has been observed that the mean dimensions of the species, within a genus, are allometrically arranged with a very great degree of accuracy so far as the species dealt with in this investigation are concerned. The group including the genera, *Cypraea*, *Erronea*, *Pustularia*, and *Talparia*, belonging to *Cypraeidae* within which they have especially related and similar forms, is also definable by means of the allometrical equation within a range of small deviations from the theoretical values.

All the specimens used in this investigation were adult forms. The constants actually calculated, therefore, do not express the true relative growth, but the directions of variation of size as will be discussed in a later section. But it is practically certain that they have some relations with the growth. The allometrical equation thus applied to the genus and to the whole group also reveals a tendency to a specific differentiation at the present stage in the process of evolution, instead of representing the conditions of relative growth. That is, although the breadth/length and height/length relations of every species show positive or negative allometrical results without reference to that of the genus to which they belong, there exists, among the genera, a general definite relationship in regard to the constant α, *viz.*

$$\alpha_{Cypraea} > \alpha_{Erronea} > \alpha_{Pustularia} \qquad \text{(A)}$$

in both the breadth/length and the height/length relations, provided that the contrary difference between *Erronea* and *Pustularia* in the breadth/length relation is probably insignificant. At the same time, regarding the constant b, an antithetical relation exists, *viz.*

$$b_{Cypraea} < b_{Erronea} < b_{Pustularia} \qquad \text{(B)}$$

Furthermore, in the breadth/length relation, these genera, in general, show a positive tendency; *viz.* the increase of breadth is greater than that of length, and in the height/length relation, *Cypraea* only shows a positive tendency and the others a negative.

HERSH found that in the evolutionary relative growth of Titanotheres[2] and in the relative growth of genetic mutants of *Drosophila*[3], b is a decreasing exponential function of α, *viz.*

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[2] HERSH, A. H. 1934. Evolutionary Relative Growth in the Titanotheres. Amer. Nat. 68, 537.

[3] HERSH, A. H. 1931. Facet Number and Genetic Growth Constants in Bar-eyed Stocks of *Drosophila*. J. Exp. Zool. 60, 213.

TABLE 2.

Species	$\alpha \pm$ P.E.	$\log b \pm$ P.E.	b
Erronea marmorata (SCHRÖTER)	1.155 ± 0.099	$\bar{1}.74293 \pm 0.00634$	0.553
	1.284 ± 0.086	*$\bar{1}.65352 \pm 0.00552$*	*0.450*
E. japonica SCHILDER	0.997 ± 0.032	$\bar{1}.77577 \pm 0.00737$	0.597
	0.999 ± 0.036	*$\bar{1}.68531 \pm 0.00852$*	*0.485*
E. artuffeli (JOUSSEAUME)	0.928 ± 0.044	$\bar{1}.79013 \pm 0.01035$	0.617
	0.892 ± 0.037	*$\bar{1}.71109 \pm 0.00865$*	*0.514*
Pustularia moneta (L.)	0.736 ± 0.106	$\bar{1}.94194 \pm 0.03235$	0.875
	0.865 ± 0.057	*$\bar{1}.71881 \pm 0.01733$*	*0.523*
P. annulus (L.)	0.789 ± 0.116	$\bar{1}.93904 \pm 0.04439$	0.869
	0.842 ± 0.065	*$\bar{1}.75320 \pm 0.02496$*	*0.567*
P. boivinii (KIENER)	0.951 ± 0.065	$\bar{1}.81243 \pm 0.03208$	0.649
	0.987 ± 0.059	*$\bar{1}.68665 \pm 0.02906$*	*0.486*
P. caputserpentis (L.)	1.227 ± 0.102	$\bar{1}.73134 \pm 0.05076$	0.539
	1.066 ± 0.124	*$\bar{1}.66703 \pm 0.06205$*	*0.465*
Trivirostra oryza (LAMARCK)	1.130 ± 0.025	$\bar{1}.87817 \pm 0.00446$	0.755
	0.931 ± 0.025	*$\bar{1}.78814 \pm 0.00440$*	*0.614*

Numerals in common type express breadth/length relations and Italic numerals height/length relations.

TABLE 3.

Genus	Breadth/Length Relation			Height/Length Relation		
	$\alpha \pm$ P.E.	$\log b \pm$ P.E.	b	$\alpha \pm$ P.E.	$\log b \pm$ P.E.	b
Cypraea	1.122 ± 0.026	$\bar{1}.70368 \pm 0.00676$	0.505	1.058 ± 0.020	$\bar{1}.65960 \pm 0.00524$	0.457
Erronea	1.004 ± 0.009	$\bar{1}.76768 \pm 0.00443$	0.586	0.972 ± 0.014	$\bar{1}.68497 \pm 0.00695$	0.484
Pustularia	1.022 ± 0.031	$\bar{1}.80647 \pm 0.01945$	0.640	0.907 ± 0.016	$\bar{1}.73107 \pm 0.00976$	0.538

TABLE 4.

Values of the whole group including the genera, Cypraea, Erronea, Pustularia, and Talparia.

Constant	Breadth/Length Relation	Height/Length Relation
α	1.057 ± 0.011	1.015 ± 0.007
$\log b$	$\bar{1}.76804 \pm 0.00665$	$\bar{1}.68127 \pm 0.00442$
b	0.586	0.480

$$b = Be^{-r\alpha} \qquad\qquad (1)$$

where B and r are constants, e being the base of the natural logarithm. This equation has been ascertained by MARCH[4] to hold good in respect of the weight/length relation of the frog. The more general form of this equation is, C being a constant,

$$b = Be^{-r\alpha} + C \qquad\qquad (2)$$

In equation (2), when $\alpha = 0$, $b = B + C$, and when $\alpha = \infty$, $b = C$. As LUMER[5] has pointed out, as regards HERSH's equation, equation (2) represents, of course, an empirical approximation without theoretical validity. This equation is derived from integrating

$$-\frac{db}{d\alpha} = rBe^{-r\alpha}$$

Therefore, the logarithm of the rate of change of b is proportional to the decrease of α. Equation (1) is a special case of $C = 0$ in (2). It was observed that equation (2) is applicable to some species of *Erronea* and *Pustularia*, viz. in the breadth/length relation

$$b = 35.9 \ e^{-0.11\alpha} + 0.520,$$

and in the height/length relation

$$b = 36.1 \ e^{-0.03\alpha} + 0.445.$$

This equation must be applicable to a group, in which many species belonging to many genera can be collected without there being inconsistency in their quantitative relations. In other words, each of these many species has a continuous phase in quantity, or shows a quantitatively successive variation from the other.
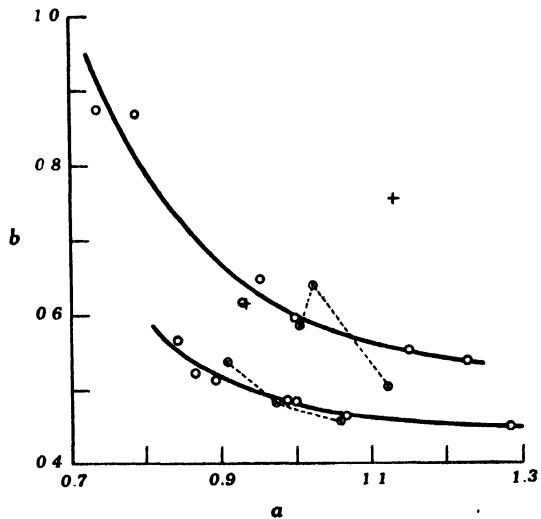
Fig. 1. *b*-α relation. ○ species, ⊣ *Trivirostra oryza* ◑ genus.

[4] MARCH, F. 1937. Relative Growth in Amphibia. Proc. Zool. Soc London. Ser. A. 107, 415.

[5] LUMER, H. 1936. The Relation between b and k in Systems of Relative Growth Function of the Form $y = bx^k$. Amer. Nat. 70, 1.

Now, the equations, actually determined here, were established from the meagre data relating to the species belonging to the two genera, *Erronea* and *Pustularia*; they must, however, have a community of application to every species in the *Cypraea-Erronea-Pustularia-Talparia* group, and that because every genus, and in fact, the whole group including these genera, is subject to the definite relation, *viz.* that expressed by the allometrical equatien. The genus *Talparia* may possibly be contained in this group. In this sense, *Trivirostra oryza*, which however seems to resemble the species in the group, must, perhaps, be excluded, since the values of *b* and *α* are, in its case, far different from those in these equations (Fig. 1), and its deviation from the theoretical size, calculated by the allometrical equation applied to the whole group, is also relatively large.

DISCUSSION

The allometrical equation applied to one species, expresses, of course, the process of ontogenetic growth, and takes for granted that the ratio of the relative growth-rates of two dimensions is approximately constant. In this case, the age or time factor is eliminated and often neglected. In a case, however, in which the time factor is neglected, the assumption that the ratio of relative growth-rates is constant, ceases to have any validity, and this equation merely shows the direction of variation in place of representing the growth process. Accordingly, in a group of a single species containing individuals of different ages, the only result arrived at by this equation is, in terms of relative growth without regard to time, how much one dimension increases, while the other, comparable with it, also increases, *i. e.* whether the ratio between the relative increases of the two is constant. When the time factor is taken into account, it is found that the ratio of relative growth-rates is not constant in many cases, but gradually changes with time[6].

In the present case, of course, the question is whether the direction of individual variations occurred as the results of growth but there are no data for ascertaining the growth process. Thus, a further hypothesis is suggested by this consideration in regard to the direction of the specific variations within a genus, being further extended to that of genus-variations within a group containing many genera, and so on. In this sense, the variation from one species to another is directed in an approximately

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[6] HAMAI, I. 1937. Some Notes on Relative Growth with Special Reference to the Growth of Limpets. Sci. Rep. Tôhoku Imp. Univ. Biol. 12, 71.

constant manner to every species. As regards the grouped genera, the same is also to be said. This fact indicates obviously an equilibrium condition in the present stage of evolution. In the present stage of phylogeny, the constant α has, therefore, a meaning as the equilibrium constant expressing the static conditions of bodily variation among various species. For the purpose of expressing this fact more completely, the b-α relation is significant. By this relation and α of the genus, the conditions of specific variation are better defined. For instance, in the group containing *Cypraea*, *Erronea*, *Pustularia*, and *Talparia*, the relative increasing rate of the breadth of the shell is on the average 1.057 times that of the length, the scale of individual variations of each species is expressed by its allometric equation, and the conditions determining the variations of species in the group containing the two genera, *Erronea* and *Pustularia*, are further limited by $b = 35.9 \ e^{-6.116} + 0.520$ in the breadth/length relation. The genera, *Cypraea* and *Talparia* are perhaps contained in this b-α equation, because it may be deduced from the fact that the group containing these four genera has a definite direction of variation, within small deviations.

As to the quantitative regularity within a group, there are some reports of the results of studies of the relation between the brain-weight and body-weight in various vertebrates, and between egg-weight and body-weight in birds. DUBOIS[7] has shown that the relation of brain-weight to body-weight in different species of various mammalian orders is expressed by the allometric equation. For instance, there exists the relation $y = 0.41$ $x^{5/9}$ in the case of *Canis*. He has expressed α as holding good from species to species as the phylogenetic exponent, and has distinguished it from the exponent holding good between the adult individuals of one and the same species, as the ontogenetic exponent. HUXLEY[8] has shown that in a selected group of birds measurements can be apparently expressed by the allometric equation in regard to egg-weight and body-weight; the equilibrium constant however gradually decreases with increasing body-weight from 1.0 to about 2/3. The difference between groups with respect to relative egg-weights remains approximately constant throughout the whole range of body-size. In the dimensions of the molluscan shells such an approximately uniform relation of the equilibrium constant as that

[7] DUBOIS, EUG. 1923. Phylogenetic and Ontogenetic Increase of the Volume of the Brain in Vertebrata. Proc. Roy. Acad. Amsterdam. 25, 230.

[8] HUXLEY, J. S. 1927. On the Relation between Egg-weight and Body-weight in Birds. J. Linn. Soc. (Zool.), 36, 457.

in ·bird-groups and in mammalian groups does not exist, but it varies with the groups, and, then, the difference between these groups with respect to relative height or breadth is not exactly constant. SHAW[9] has recently shown that in the Chinese birds groups, Gruiformes and Passeriformes, the relation between egg-weight and body-weight is expressed by this formula and the value of α is approximately 2/3 or a little more than that, but less than 1.0, as already shown by HUXLEY. That of b is different in every bird's order, although the species here dealt with are few.

HERSH[2] has applied the allometric equation to the genera of Titanotheres, and summarized the results in the following tentative generalizations. (1) Ascending species within the genus bring about no change in b and α; (2) genera are distinguished from one another by differences in b and in α; (3) b is a decreasing exponential function of α. He has also proved the third conclusion in regard to the various mutants of *Drosophila*[3]. These conclusions are generally recognizable in the case of the group *Erronea* and the others. But in the application of the hypothesis of growth-partition to the group the fact that every species undergoes a different growth-partition is apt to be overlooked. Strictly speaking, the relations in regard to the group are average results, and give the distributional conditions of variation, which the specific growth-partition exhibits. The group-data are not to be expressed in terms of the ontogenetical growth-relations, but in those of the phylogenetical or mutational directions or equilibria.

FRANZ[10] has elucidated the significance of the constants m and n in the snail *Viviparus* by the equation, $B=mH+n$, B being the breadth and H the height, but showing the fact that the complete curve, including the young of less than 10 mm. in height, is probably a parabola; in practice, however, this equation has been sufficient to express the change of form. That is, he says that, phylogenetically, on the whole, m, the coefficient of H, diminishes, and the constant term n increases; in other words, the shell passes more and more from the cone-shape to the barrel-shape (upper half of a barrel), in which the older individuals are more slender in proportion to height than the younger. This fact has been

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   [2] HERSH, A. H. 1934. *loc. cit.*

   [3] HERSH, A. H. 1931. *loc. cit.*

   [9] SHAW, TSEN-HWANG, 1937. Notulae circa Sinensis Aves. (1) Egg-weight and Body-weight in Some Chinese Birds. Bull. Fan Mem. Inst. Biol. (Zool.), 7, 187.

   [10] FRANZ, V. 1935. Results in the Phylogeny of a Genus of Snails. Quar. Rev. Biol. 10, 80.

ascertained chronologically, and it shows, therefore, the temporal direction of evolution, *viz.* phylogenetic relative growth. On the other hand, the data of *Cypraea* and the others show the static state of evolutional results in a period, *viz.* the directions of the distribution of variation.

It may not be necessary to interpret the bodily changes, definitely directed as mentioned above, by the conception of orthogenesis, as pointed out by HUXLEY[11] in regard to the increase of size and the emergence of horn, of Titanotheres. These changes may be better accounted for by the operation of natural selection on the growth-gradient, which gives a definite mode of change in size and in form, and which are latent poten-tialities of growth as a quantitative factor in the germ-plasm. That is, it is proved that a quantitative change evokes a qualitative one. Now, in the case of the group of *Cypraea* and the others, the phylogenetical growth-gradient is conditioned by the factors expressed by the allometric equation and by equation (2) within the limited scale. The relations, (A) and (B), may be parallel phenomena with size-limitation, *viz.* *Cypraea* has a strikingly larger size than have the others; the difference in size between *Erronea* and *Pustularia* is small, but the largest species of *Erronea* is larger than the largest of *Pustularia*. The growth-gradients of each species are serially arranged within a genus according to the absolute size, if they are judged by reference to the specific values of α. Thus, these growth-factors will affect the size-limitation. For instance, in *Pustularia*, the growth-partition in the direction radial to the longitudinal axis (length) increases with the average body size, but in *Erronea* this condition is the reverse.

SUMMARY

As results of the above arguments regarding on *Cypraea, Erronea, Pustularia, Talparia,* and *Trivirostra,* the following conclusions are sum-marized : —

(1) The allometric equation can be applied to a genus and to a group including the genera, *Cypraea, Erronea, Pustularia,* and *Talparia.*

(2) Among the given genus-group in *Cypraeidae,* the following relations appear to exist.

$$\alpha_{Cypraea} > \alpha_{Erronea} > \alpha_{Pustularia}$$

$$b_{Cypraea} < b_{Erronea} < b_{Pustularia}$$

11) HUXLEY, J. S. 1932. Problems of Relative Growth. London.

in both the breadth/length and height/length relations.

(3) b and α are empirically expressed by the exponential function, $b = Be^{-r\alpha} + C$, in some species of *Erronea* and *Pustularia*, where B, r and C are constants and e the base of the natural logarithm; and this relation will be further extended to all the species in the group in question.

(4) The values of α and b of every species in the selected group are correlated with the body-size, *viz.* in *Erronea*, the smaller the body-size, the greater α and the smaller b has its species; on the other hand, in *Pustularia*, the relationships are quite the reverse.

(5) Such a relation may also exist between the genera, *viz.* there exists the relation, *Cypraea* > *Erronea* > *Pustularia*, in the body-size, in correlation with the second conclusions.

STUDIES ON THE BODY FLUID OF AN ASCIDIAN, CHELYOSOMA SIBOJA OKA WITH SPECIAL REFERENCE TO ITS BLOOD SYSTEM[1]

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INTRODUCTION

The studies of the acidic reaction of the ascidians, have hitherto been made on the body fluid from the different tissues as in the blood and in the mantle of some limited species, found in the Mediterranean Sea and the Atlantic Ocean.

The first description of the presence of this reaction was given by HENZE (1911, 1912) in the blood of *Phallusia mammilata* at Naples. He made chemical analysis of the plasma and the corpuscles separated from its blood, together with that of the surrounding sea water.

From the fact that the corpuscle shows the higher value of $\frac{SO_3}{Cl}$ than the plasma and the sea water, he concluded that the acid reaction was caused by the presence of free sulphuric acid contained in the corpuscles (about 3%), but not in the plasma. HENZE (1913₁) classified the blood corpuscles into the two groups, the round and ellipsoidal cells with large diameter, and the mulberry-formed cells with the green coloured granule.

By means of the various tests, specially of the staining reaction with the neutral red and other dyes, the mulberry-formed cells was only the container of free sulphuric acid which coexists with the green pigment in the same cell.

He (1913₂) further reported that sulphuric acid was obtained by pressing the mantle tissue of another species of ascidian *Ascidia mentula*, but could not detect sulphuric acid in the mantle tissue of *Phallusia mammilata*.

HECHT (1917) demonstrated the presence of the acid reaction in the green cells among the blood cells of *Ascidia atra* at the Bermuda Islands in the Atlantic Ocean. This reaction was only detectable by means of

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litmus paper under the microscope, probably owing to the insufficiently small quantity of the blood fluid, as was found in the case of *Phallusia mammilata*.

Among these ascidian species so far studied by others, the acid reactions appear to occur in the different parts of the body according to species, being distinguishable into two types; one as found in the special corpuscle cells of the blood both in *Phallusia mammilata* and *Ascidia atra*, while the other in *Ascidia mentula* it occurs in the bladder or vesicular cells of the mantle tissue.

The present writer (1933) has found not only the similar acid reaction, in one of the ascidians, *Chelyosoma siboja* OKA, living in Mutsu Bay, but even free sulphuric acid was detected in the body fluid obtained from the cut source of the test.

The acid reaction was chiefly testified by the chemical analysis of anions related to its acid radicals, SO_4, Cl, and PO_4, as well as by the electrometric titration with 0.1 N sodium hydroxide solution of the body fluid, the plasma and the corpuscle fluid. The amount of sulphuric acid was estimated at 4.3% in the corpuscle fluid and 1.3% in the plasma fluid.

My subsequent chemical analysis of these three fluids and of the surrounding sea water (1935), revealed the fact that the general feature of the ionic distribution of the corpuscle fluid is remarkably different from those of the plasma fluid and the sea water, and the greater fraction of SO_4 is highly accumulated in the corpuscle fluid, in spite of the fact that sodium and chlorine are largely predominant in the surrounding media, the plasma and the sea water.

Furthermore the corpuscle cells of *Chelyosoma siboja*, which gives the acid reaction differ more or less from those of the other ascidian species, studied by HENZE and HECHT, in the occurrence of the two sized cells: larger cells which measure 50 μ in diameter and smaller which measure 10 μ in diameter (1933).

The purpose of the present work was to anatomically locate the test fluid in the ascidian body, and also in particular cells among various corpuscle cells contained in the body fluid by means of the staining dyes.

The chemical analyses of the conjugated sulphates in the corpuscle fluid and of the inorganic composition of the perivisceral fluid were also performed in this experiment. This work was carried out partly at the laboratory of the Marine Biological Station of Asamushi, and continued at the zoological laboratory of this college.

METHOD

The freshly collected animals, cleaned from the adhering mud, were kept in the three litres glass bowl and stained for three or four days with neutral red dye which was previously dissolved in the sea water. Then the animal was cut at the surface of the test, the test fluid delivered, was collected in the centrifuge tube by gently pressing of the whole ascidian body. Immediately after this treatment, the soft body was carefully removed from its test by cutting the joint of the syphone plate, and the blood fluid was collected from its heart vessel by inserting the syringe needle.

Both test fluid and blood fluid thus collected were separately centrifugarized and the sediment was used for the detection of the corpuscle cells exhibiting the acid or non-acid reaction and also for the enumeration of these cells. For the enumeration of the acid and non-acid cerpuscle cells, the Thoma-Zeiss' haemozytometer was employed. At the same time, the cartilaginous test just removed from the ascidian body was both macroscopically and microscopically used for the observation of the anatomical relation of the body fluid between the test and soft body, and for the distribution of the acid reaction.

Under the microscope, the other dyes beside neutral red, such as methyl red and methyl violet were also chosen for the staining reaction of the acid cells.

For the quantitative determination of the conjugated forms of sulphate and unoxidized sulphur in the corpuscle fluid, each test and blood fluid was collected from the samples of 50 individuals in the same way as in the case of the vital staining of neutral red dye. After centrifugarization of these fluids at 3000 rotations per minute, each separated corpuscle was frozen by the same way, as was applied in my previous works (1933, 1935), and used for the chemical analysis of sulphate.

Sulphates in the various forms were determined gravimetrically as the precipitate of barium sulphate. Inorganic sulphate directly precipitates with 5% solution of barium chloride. 2-4 cc. of each sample fluid was taken in a 200 cc. Ehlenmayer flask, the content was diluted to 150 cc. by adding distilled water, and 10 cc. of the above barium chloride solution was slowly added. After allowing this mixture to stand for one hour, the precipitate of barium sulphate thus formed was filtered, then washed with about 250 cc. of water on the filter paper, finally ignited in a platinum crucible and weighed.

The etheral sulphate determination was made by boiling in the presence of the mineral acid. The diluted hydrochloric acid (one volume of the concentrated hydrochloric acid to four volumes of water) was added to the same volume of the corpuscle fluid from which the masked sulphate was freed by the hydrolysis of the organic substance, then sulphate was precipitated by barium chloride solution.

Unoxidized sulphur was determined by the preliminary evaporation of water and the organic substances ignited off at the presence of the BENEDICT's sulphur reagent, then sulphate was precipitated, filtered, ignited and weighed. The acid sulphate was determined by the titration of 0.1 N sodium hydroxide solution and calculated as the acid sulphate.

The analysis of the perivisceral fluid was made by the following methods, sodium by the method of KRAMER and GITTERMAN (1924), potassium by the KRAMER and TISDALL method (1921), calcium by CLARK-COLIP modification of the KRAMER and TISDALL method (1925), magnesium by DENIS method (1922). Chlorine by WHITEHORN method (1921), sulphate by KAHN and LEIBOFF method (1928). The benzidine sulphate formed by this method was titrated volumetrically with 0.1 N sodium hydroxide solution by phenolphthalein instead of by the colorimetry described by the above workers.

For the removal of protein in the perivisceral fluid, tungustic acid by the method of FOLIN and WU, or 20% trichloracetic acid were employed in this experiment. The sea water collected in the surrounding of the ascidians, was analysed for the comparison; and the technique and the method of the analysis were the same as those applied in my previous works (1933, 1935). For the determination of the freezing point depression, the Beckman's thermometer was employed and the pH was measured by the indicators, phenol red and cresol red.

EXPERIMENTAL RESULTS

The Staining Reaction by the Vital and Other Dyes. After two or three days since the animals were immersed in the sea water, in which the vital dye, neutral red was previously dissolved, though fully well stained, the corpuscle and other animal tissues were actively alive even more than a week.

It was found that the fluid obtained from the ascidian test was deeply stained in wine red colour *in vivo*, showing clear evidence of the acid reaction. Inside the dissected and removed cartilaginous test, the acid reaction could easily be observed at the test channels where the test

vessel was penetrated, being anastomosically distributed along the outer margin of the cartilaginous test. These test vessel were connected only at one point through the blood sinus located at the ventral side of the test into the blood vessel of the soft body.

On centrifugarization of the test fluid, it was noted that the separated corpuscles at the bottom of the centrifuge tube were stained in the deep wine red colour, while the upper layer of the plasma fluid exhibited much weaker acid colour reaction than that of the corpuscles.

The similar colour reaction was also observed in the blood fluid obtained from the heart vessel when these were treated with the same method as was applied to the test fluid.

Recently OHUYE (1936) made morphological studies of the blood of several species of ascidians living in Mutsu Bay. According to his report, the blood of *Chelyosoma siboja* OKA contains many different types of corpuscle cells such as the green cell, the brown cell, the hyaline amoeboid cell, the coarsely granular amoeboid cell, the vesicular cell, the vesicular amoeboid cell, and the compartmental cell. He further noticed that the vesicular cells are most abundant in the blood of *Chelyosoma siboja.*

The general morphological characteristics of each corpuscle cells in the test fluid are identical with those in the blood fluid as was already described by OHUYE (1936). In this experiment, however, I found it more convenient to group those into two types according to their size, i. e. the large vesicular cell with 45-60 μ in diameter and the small wandering cell types with 7-10 μ diameter. The former was found to be highly enlarged and vacuolated, being surrounded by the thin protoplasmic membrane. A minute green granule was observed, close to its nucleus while the vesicular amoeboid cell contained one or several green granules. In the majority of cases the cell sap of the vesicular cell was stained pink colour but occasionally deep wine red colour by the vital staining of neutral red. A deep wine red colour appears in most of the vesicular amoeboid cell, but rarely in the vesicular cells, though cell saps stain in both kinds of the vesicular type cells.

In the younger stage of these cells, several forms of vacuoles with the acid reaction are sometimes observed, the fact which probably suggests the gradual development of its cell sap. The diameters of these young amoeboid cells range 7-30 μ but mostly 10-15 μ.

The remaining other kinds of cells such as the green cell, the brown cell, the hyaline amoeboid cell, the coarsely granular amoeboid cell, and the compartmental cell fail to exhibit the acid reaction by staining. The

diameter of these cells measures 7–10 μ, except the compartmental cells which measure 10–16 μ in length.

The acid reaction in the cell sap of the vesicular type cells is also demonstrable by means of methyl red, as was employed by HENZE (1912) in the blood of *Phallusia mammilata*. The similar acid reaction is obtained by methyl violet which stains yellowish in colour indicating that the hydrogen ion concentration in the cell sap may be as low as pH 1.0. The external medium of the vesicular cell under the same conditions stains greenish indicating much lower acidity than that of the cell sap. The yellow colour produced by methyl violet closely agrees with my previous work, electrometrically determined on the frozen corpuscle fluid, which gave pH 0.38 (KOBAYASHI, 1933).

On the other hand, in the cartilaginous test, neither trace of acid nor cellular element was detected microscopically from the slice of test tissue.

It is concluded from the above experiment that free acid was produced *in vivo* in the cell sap of the vesicular type cell in the body fluid (test fluid and blood fluid) of *Chelyosoma siboja* but not in the cartilaginous test tissue.

Enumeration of the Acid and Non-acid Corpuscle cells in the Test and Blood Fluids. The two types of the cells, i. e. the acid cells (the vesicular type cells) and non-acid cells (the remaining other cells except the vesicular type cell), are distinguishable in the body fluids of *Chelyosoma siboja* by the vital staining or by other dyes, and the results of the enumeration of these are shown in the following table.

The table shows that the number of the vesicular cells giving the acid reaction are nearly equal to the number of the non-acid cells in both the test and body fluids; 46–48% of the total number of the cells.

The number of the vesicular amoeboid cell is respectively 7% of total and 14–15% of the remaining other corpuscle cells. The similar percentage values were obtained in both the test and blood fluids.

Sulphate and Sulphur of the Various Forms in Conjugation. The amount of sulphate in the etheral form which is loosely combined with the organic substances, and sulphur in the unoxidized form, were determined with the corpuscles fluids obtained from the test and the blood. In addition sulphate in relation to the sulphuric acid, in the inorganic non-acid form and chlorine were determined for the purpose of the comparison of the conjugated sulphates.

TABLE 1.

*Number of the acid and non-acid corpuscle cells
in the test and blood fluids.*

Individuals	Test fluid			Blood fluid		
	Acid cell		Non-acid cell[1]	Acid cell		Non-acid cell
	Vesicular cell	Vesicular amoeboid cell	Remaining other cell	Vesicular cell	Vesicular amoeboid cell	Remaining other cell
1.	408	45	380	411	72	506
2.	398	59	439	409	47	402
3.	397	40	337	396	52	364
4.	396	47	389	390	62	351
5.	397	40	337	396	52	364
Total	1984	276	1982	1991	304	1999

Percentage of the acid and non-acid cells for
the total number of these cells.

47.8	6.9	45.6	46.4	7.0	46.6

Percentage of the vesicular amoeboid cells for the
remaining other cells

—	14.0	—	—	15.2	—

TABLE 2.

*Acid, non-acid, and ethreal sulphates, unoxidized sulphur and
chlorine content in the corpuscle fluid.* (gm. in 100 cc.)

Fluid	Total sulphate			Unoxidized sulphur	Cl
	Inorganic sulphate		Ethreal SO_4		
	Acid SO_4	Non-acid SO_4			
Test	3.34	0.26	0.02	0.01	0.34
Blood	3.39	0.31	0.04	0.01	0.27

As the table shows, the analytical values of the conjugated forms of sulphate and sulphur in the same series from the test fluid are almost nearly identical with those corresponding value in the blood fluid.

Among these forms of sulphate and sulphur, the greater fraction of sulphate is found to be in the inorganic state, related to the sulphuric acid which reaches 92.3% in the test fluid and 90.6% in the blood fluid

of the total amount of sulphate. 7.1% in the test fluid and 8.5% in the blood fluid are non-acid sulphate. The amount of the conjugated ethreal sulphate, and of sulphur in the unoxidized form are found to be extremely lower than those of inorganic acid and non-acid sulphates.

Inorganic Constituent of the Perivisceral Fluid. In this experiment, the perivisceral fluid which surrounds the heart of the ascidian soft body, was analysed together with the sea water. The results obtained are shown in Table 3.

TABLE   3.

*Inorganic composition of ascidian perivisceral fluid
and of the Asamushi sea water.*

	Gram in 1000 cc.		Gram ionic concentration		Gram ionic proportion	
	Asamushi sea water	Perivisceral fluid	Asamushi sea water	Perivisceral fluid	Asamushi sea water	Perivisceral fluid
Cl	18.44	18.33	0.520	0.517	100.00	100.00
Na	10.40	10.10	0.452	0.439	86.92	84.91
K	0.39	0.50	0.010	0.013	1.92	2.52
Ca	0.411	0.402	0.011	0.010	2.12	1.93
Mg	1.15	0.95	0.047	0.039	9.04	7.54
SO$_4$	2.70	2.09	0.028	0.021	5.39	4.16
pH	8.2	7.2				
Δ	1.97	1.96				
			1.068 (Total)	1.039 (Total)		

As will be seen in the above table, the inorganic composition of this fluid was nearly identical with that of the sea water, though the slight difference is found in the content of sulphate, magnesium, and potassium, being slightly lower in the content of sulphate, and higher in the potassium content, as compared with those of the sea water.

The freezing point depression of this fluid was determined to be 1.95, showing almost the same value as that of the sea water.

The value of pH 7.2 of the perivisceral fluid differs from pH 8.2 of the sea water.

CONCLUSION

From the results obtained by the vital staining and the staining reactions with other dyes, neither trace of the acid reaction nor the

cellular element is present in the cartilaginous test in which the test vessels are densely anastomosed. The test vessels are anatomically continuous through the blood sinus to the blood system of the soft body at one point of the ventral side of the ascidian body.

This anatomical connection between the test and the blood vessels, is further ascertained from the fact that the numbers of the corpuscles, such as the vescicular cell, the vescicular amoeboid cell and the remaining other cells, in both vessels are almost equal, and in addition the amount of sulphates and sulphur in the various forms are practically the same in these two different vessels.

Microscopically it was found that free sulphuric acid is highly accumulated in the cell sap of the vesicular cells (48–60 μ in diameter) and in the vesicular amoeboid cells (10–30 μ in diameter), while the other remaining cells, the green cells, the brown cells, the hyaline amoeboid cells (7–10 μ in diameter), and the compartmental cells fail to present the acid reaction.

The hydrogen ion concentration of the cell sap of the vesicular cell is estimated from the staining reaction to be lower than pH 1.0.

This estimated value appears to approach closer to the value (pH 0.38) determined by the electrometric method from the frozen corpuscle fluid (KOBAYASHI, 1935).

In both the test and blood fluids, the number of the vesicular cells is 46–48% of the total number of the corpuscle cells, thus being almost equal to that of the other remaining non-acid cells. The number of the vesicular amoeboid cell is small being only 7% of the total.

In regard to the volume ratio between the acid cells and the non-acid cells in the ascidian body fluid, if we assume that the average diameter of the vesicular cell is 50 μ and that of the other remaining cells is 10 μ and since the volume of sphere is proportional to the cubic root of the length of its radius, the relation of $\frac{\text{Other remaining cell}}{\text{Vesicular cell}}$ becomes $\frac{1}{125}$, or less than 1%. From the enormous volume as well as multitudinous number of the vesicular cells in the body fluid, it becomes obvious that free sulphuric acid stored in the cell sap of the vesicular cell seems to be the chief source of the high amount of sulphuric acid in the corpuscle fluid (4.3%).

The fact that a trace of the conjugated sulphate and unoxidized sulphur in the corpuscle fluid is detected in both the test and blood fluids, suggests that the majority of sulphate (98% of total sulphate) may exist in the inorganic state, chiefly in the form of free sulphuric acid (90% of

total sulphate) and the remaining (8% of total sulphate) in the non-acid inorganic form.

The inorganic composition of the perivisceral fluid which surrounds the ascidian heart, is found to be nearly the same as that of the sea water, but remarkably differs from the data given by the present writer on the composition of the blood fluid (body fluid), specially of the corpuscle fluid (KOBAYASHI, 1935).

It is interesting to note that in both *Phallusia mammilata* and *Ascidia atra*, the acid reaction is present only in the green cell, one of the blood cells with the green granules while in *Ascidia mentula* the vesicular cell embedded in the mantle tissue, is considered to be the container of sulphuric acid. In *Chelyosoma siboja*, however, the green granule which is analogous to the green granules of *Phallusia mammilata* and *Ascidia mentula* is contained in the vesicular type of the cells.

SUMMARY

In *Chelyosoma siboja* OKA, the body fluid in the test originates from the blood vessels distributed in the test and in the soft body. The vessels in the test are continuous to the blood system of the soft body, through the blood sinus located at the ventral side of the ascidian body.

The vesicular cells seem to be the chief source of the acid reaction, especially in the cell sap in which free sulphuric acid is highly accumulated.

Sulphate in the corpuscle cell is found to be in the form of inorganic, chiefly in the state of sulphuric acid.

Inorganic composition of the perivisceral fluid almost resembles that of the sea water, being more or less different in the content of potassium, magnesium and sulphate, but remarkably differs from the data on the composition of the blood fluid (body fiuid), specially of the corpuscle fluid (KOBAYASHI, 1935).

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REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY
32. MEDUSAE FROM MUTSU BAY (REVISED REPORT)[1]

BY

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Since the publication of the first report on the medusae from Mutsu Bay in 1927, several forms which were not referred to at that time, have been collected from the Bay and kindly sent to the writer for identification by Dr. S. KOKUBO and other gentlemen of the Asamushi Marine Biological Station. A list of all the medusae hitherto found there will be given in the following, with some short additional notes. Medusae treated in this paper are mostly forms of the temperate waters or cosmopolitic species, but the seven species; *Sarsia tubulosa, Hybocodon prolifer, Catablema multicirrata, Willsia flavicirrata, Melicertum octocostatum, Aglantha digitale* and *Cyanea capillata*, are circumboreal in distribution.

Hydromedusae

Order ANTHOMEDUSAE
Family CODONIIDAE

Sarsia tubulosa (SARS)

Sarsia mirabilis: UCHIDA, 1927, pp. 179–180; — 1930 p. 330.

The species was formerly described as the American medusa, *S. mirabilis*, which was definitely identified with this species by KRAMP (1927) and THIEL (1932). The medusa is frequently collected on the coasts of Hokkaido from spring to summer. Several specimens of this species were found in surface tows on April 30, 1929.

Hydrocoryne miurensis STECHOW

Hydrocoryne miurensis: STECHOW, 1909, pp. 35–39; UCHIDA, 1932, pp. 135–138.

This species was described on the basis of the polyp generation by STECHOW (1908 & 1909). The medusa form was described by the present

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[1] Contribution from the Marine Biological Station, Asamushi, Aomori-ken, No. 151.

writer on young medusae liberated from the hydroids collected in July
1929 in Mutsu Bay. Medusae about cubic in shape on account of four
well developed radial canals, 1–1.5 mm high and 0.8–1.2 mm wide.
Mesogloea rather thin but rigid. Exumbrella sprinkled with remarkable
nematocyst clusters. Four radial canals straight and broad, opening into
a large stomach cavity. A ring canal straight. A large tentacle bulb,
occurring at the base of the radial canals, is provided with thick nematocyst
layers on the axial side and a large dark brown ocellus on the abaxial
side. Tentacles hollow, broad near the base but generally narrowing to
the terminal portion. Nematocyst clusters of the tentacles conspicuous in
the main shaft except at the basal portion where only a few of them are
scattered. Velum comparatively narrow. Manubrium flask-shaped and
reaching midway the bell down the bell cavity, provided with a round
mouth. In newly liberated medusae an apical canal, the remnant of
budding, extends upwards from the stomach cavity, opening outside.
Gonads not yet developed in these specimens. Manubrium slightly brown,
ocelli and radial canals dark brown.

Localities. This hydroid seems to be rather common on Japanese
coasts since it has been found in Misaki, Asamushi, Akkeshi, Onagawa
Bay and Shimoda, Shizuoka Pref.

Family TUBULARIIDAE Hincks, 1868 emend Uchida, 1927.

Euphysa sp.

Euphysa sp.: Uchida, 1927, p. 217.

A single specimen was collected on March 14, 1926.

Hybocodon prolifer L. Agassiz

Hybocodon prolifer: Uchida, 1927 (a), pp. 217–218; —.1927 (b), pp. 162–163 & pp. 192–193.

The species is one of the cold water forms. It is common on coasts
from Hokkaido to Kamchatka. The development of the eggs to actinulae
on the manubrium of the mother medusa was described in the former
paper (1927, b, pp. 162–163). Medusae and actinulae are not rare in
early spring.

Family CLADONEMATIDAE Gegenbaur, 1885.

Cladonema radiatum var. mayeri Perkins

Cladonema radiatum var. *mayeri:* Uchida, 1927 (a), p. 218 —, 1927 (b), pp. 197–198.

Cladonema mayeri: KRAMP, 1928, pp. 43–45.

This medusa is a form of the European medusä, *Cladonema rediatum* with hexamerous symmetry. KRAMP (1928) described the Japanese medusa as a species distinct from the European species. Recently WEILL (Bull. Biol. France et Belg., T. 71, pp. 438–465) having studied on specimens from the Bermudas, came to the conclusion that the three species of *Cladonema:* i. e. *C. radiatum, C. perkins* and *C. mayeri*, must be merged into the first species *radiatum*. This species is common in summer in Japan from the Kurile Islands southwards to Misaki.

Family TIARIDAE HAECKEL, 1879

Urashimea globosa KISHINOUYE

Urashimea globosa: UCHIDA, 1927 (a), pp. 218-219, —, 1927 (b), pp. 205-207; —. 1930, p. 330; THIEL, 1932, p. 138.

This medusa is widely distributed on the Pacific coasts of Japan; from Saghalien, through Hokkaido to the middle part of Honshu.

Leuckartiara octona (FLEMING)

Leuckartiara octona: UCHIDA, 1927 (b), pp. 211-212. —. 1930, p. 331.

This medusa is a form of temperate oceans and was occasionally collected on the southern coasts of Honshu and Kyushu. A specimen was found in Mutsu Bay in February, 1927. This medusa was further obtained in Akkeshi Bay, Hokkaido last autumn.

Catablema multicirrata KISHINOUYE

Catablemna multicirrata: UCHIDA, 1927, p. 213; —, 1930, p. 331; THIEL, 1932, p. 136; UCHIDA, 1933, p. 130.

This medusa is a boreal form and was caught in Japan in the following localities; Kurile Islands, Hokkaido and Mutsu Bay. This species is further known near Kamchatka, Unalaska Island and Greenland. Examples found in the Bay harboured young forms of the actinian, *Peachia quinquecapitata.*

Family CYTAEIDAE

Podocoryne simplex KRAMP

Podocoryne simplex: KRAMP, 1928, pp. 45-47; UCHIDA, 1930, p. 331.

Several young medusae were collected in Mutsu Bay in April, 1926.

They are furnished with two opposite tentacles on the bell margin and four medusa buds on the manubrium. This species is likewise known from Misaki and the coast of Yamagata Pref.

Turritopsis nutricula Mc CRADY

Turritopsis nutricula: UCHIDA, 1927 (a), p. 219; —, 1927 (b), pp. 217–218. —. 1930, pp. 331–334; —, LING, 1937 (a), pp. 354–355.
Turritopsis pacifica: KRAMP, 1928, pp. 53–54.

The identification of *T. pacifica* with *T. nutricula* was discussed by the present writer in 1930 after examining the type specimens of the former species studied by the first investigator, O. MAAS. According to LING (1937) this medusa is distributed in the Pacific southwards to the Chekiang coast.

Family BOUGAINVILLIIDAE

Rathkea octopunctata (M. SARS)

Rathkea octopunctata: UCHIDA, 1927 (b), pp. 224–225; — , 1930, p. 334; —. 1933, p. 130.
Rathkea blumenbachi: UCHIDA, 1927 (a), p. 219.

In the first report on the medusae from Mutsu Bay the present writer reported this medusa as *R. blumenbachi*. As to the synonymy of the medusa, KRAMP (1926, Danish Ingolf Ex., vol. 5, pp. 58–66) gave an important account. This species was found also from the coasts of Kamchatka and Korea.

Family SPIROCODONIDAE

Spirocodon saltatrix (TILESIUS)

Spirocodon saltatrix: UCHIDA, 1927 (a), pp. 219–220; —, 1927 (b), pp. 230–233; KRAMP, 1928, pp. 61–62.

This large beautiful anthomedusa is common in Japanese waters from Mutsu Bay southwards to Kyushu, but is not known from any other locality.

Family WILLIIDAE

Willsia flavicirrata (BRANDT)

Willia stellata: UCHIDA, 1927 (b), pp. 235–236. *Willsia stellata;* UCHIDA, 1930, pp. 334–335.

Willia pacifica: UCHINA, 1927, p. 236.
Willsia pacifica: UCHIDA, 1930, pp. 334-335.

This medusa is variable in the number of the primary radial canals. The Japanese medusae described by MAAS and UCHIDA as *W. pacifica* and *W. stellata* are probably united into the medusa described by BRANDT (1838) as *Proboscidactyla flavicirrata.*

This medusa is common on the coasts of Hokkaido in summer. Several specimens were found in Mutsu Bay on June 26, 1928 and July 17, 1928.

LEPTOMEDUSAE

Family LAFOEIDAE

Melicertum octocostatum (M. SARS)

Melicertum octocostatum: KRAMP, 1919, pp. 52-57; —, 1933, pp. 237-239; RANSON, 1933, p. 316-318.
Melicertum campanula: UCHIDA, 1927 (a), pp. 220-221; KRAMP, 1933, pp. 237-239; THIEL, 1932, p. 143.

In 1933 KRAMP, who examined both European species *octocostatum* and the American species *campanula*, came to the conclusion that *M. campanula* is merely a somewhat larger growth-form of *M. octocostatum.* The Japanese medusae, though deficient in the longitudinal lines of nematocyst-like cells on the subumbrella, have been here identified with *M. octocostatum* following KRAMP's proposition. On July 11, 1929 six specimens of this species were collected at Muroran, Hokkaido. Most of these specimens lack the subumbrellar longitudinal lines of nematocyst-like cells. A single comparatively young medusa has four or five dotted longitudinal subumbrellar lines, but these lines are not distinct and are quite different from radial canals in appearance.

Distribution. Northern parts of the Atlantic, on European and American coasts.

Family EUCOPIDAE

Eucheilota paradoxica MAYER

A single specimen probably belonging to this species was obtained in Mutsu Bay in August, 1921. It agrees exactly with MAYER's description. This medusa is often found in the summer months at Misaki..

Obelia sp.

Medusae of *Obelia* are frequently observed in plankton from the Pacific coasts of Japan, but the specific identification of most of them is difficult without any knowledge of their hydroids.

Phialidium discoida (MAYER)

Phialidium discoida: UCHIDA, 1927 (b), pp. 221–222.

The medusa is frequently collected on the coasts of Honshu from the later part of summer to the early part of autumn. The young medusae of this species, which are provided with only eight tentacles, four perradial and four interradial, bear already well developed round gonads on their radial canals.

Family MESONEMIDAE

Aequorea coerulescens (BRANDT)

Aequorea coerulescens: UCHIDA, 1927 (a), pp. 222–224.

This species is a warm water form. It reaches nearly 150 mm in diameter. The distribution of this medusa in Japan is around Honshu. Specimens from Mutsu Bay were observed to harbour large young forms of the actinian *Peachia quinquecapitata.*

Family OLINDIADAE

Gonionemus oshoro UCHIDA

Gonionemus oshoro: UCHIDA, 1929, pp. 359–360.
Conionemus murbachi (in part): BROCH, 1929, pp. 488–389.
Gonionema murbachi var. *oshoro:* UCHIDA, 1927 (a), pp. 224–225.

This species is distributed around Hokkaido and on northern coasts of Honshu. It is found among Sargassum during July-August and is injurious to sea-bathers on account of its nematocysts. The present author described the medusa as a variety of the American species, *G. murbachi* but afterwards separated it as a distinct species. BROCH states that the species is related to *G. vertens* rather than to *G. murbachi* but is different from the former in the form of the gonads. Reading UCHIDA's paper (1929) he wrote personally that the institution of *G. oshoro* as a distinct species seems to be more right than to keep it as a variety of *G. murbachi.*

TRACHOMEDUSAE

Family TRACHYNEMATIDAE

Aglantha digitale MÜLLER

Aglantha digitale : UCHIDA, 1927 (a), p. 225; —, 1928, p. 79; —, 1930, p. 335; THIEL, 1982, p. 154; UCHIDA, 1933, p. 132.

The medusa is one of the boreal forms and is common in the waters of Hokkaido and the Kurile Islands. The species is known in Japan southwards to Misaki in the Pacific and off Fukui Pref. in the Japan Sea.

Family GERYONIIDAE

Liriope tetraphylla (CHAMISSO et EYSENHARDT)

Liriope tetraphylla : UCHIDA, 1928, pp. 82–83.

This medusa is common around Honshu and southern parts of Hokkaido. Large examples are usually obtained in winter in southern parts of Honshu.

Scyphomedusae

CUBOMEDUSAE

Charybdea rastonii HAACKE

Charybdea rastonii : UCHIDA, 1927 (a), p. 226; —, 1929, pp. 157-172.

The detailed description of this medusa was given by the present writer (1929). The species appears in Japan in September in bays of Honshu and Kyushu, especially in warm waters. It is further known in the Japan Sea from points on the western coast of Hokkaido such as Oshoro and Rumoé. Recently THIEL (1936, in BRONN's Klassen u. Ordnungen, Bd. 2) examined the Japanese medusa sent by the writer and reduced the species of the genus to the single Mediterranean species *C. marsupialis*. Though his conclusion is very probably correct, it seems to the present writer to be a little premature, because the former species has not been collected in such great numbers as the Japanese species and its metamorphosis has not been studied. The form of gonads, pedalia and velar canals is variable according to growth stage.

STAUROMEDUSAE

Stenoscyphus inabai (KISHINOUYE)

Stenoscyphus inabai: UCHIDA, 1929, pp. 107-112.

By the kindness of Prof. S. HÔZAWA the author could examine several specimens of this stalked medusa of warm waters, which were collected in August, 1931 in Mutsu Bay. This species has been recorded only from the coasts of Honshu and Kyushu.

Haliclystus auricula CLARK

Haliclystus auricula: UCHIDA, 1927 (a), pp. 226-228; ---, 1929, pp. 113-130; LING, 1937 (b), pp. 6-10.

This medusa is common in Mutsu Bay and the southern and western coasts of Hokkaido, but it is occasionally found, though not large in numbers, on several coasts of Honshu and Shikoku. Recently LING recorded the species from Tsingtao, China.

Sasakiella cruciformis OKUBO

Sasakiella cruciformis: UCHIDA, 1927 (a), pp. 228-229; ---, 1929, pp. 140-149; LING, 1937 (b , pp. 16-25.

Detailed notes of the medusa were given by UCHIDA (1929). Recently LING reported the species from Tsingtao, China and described several abnormal forms. The species is hitherto known in Japan from southern parts of Hokkaido (Oshoro and Muroran) and northern parts of Honshu (Mutsu Bay and Onagawa Bay).

SEMAEOSTOMAE

Family PELAGIDAE

Dactylometra pacifica GOETTE

Dactylometra pacifica: UCHIDA, 1927 (a), pp. 229-230; ---, 1935, p. 44.

The medusa is one of the commonest medusae in Japanese waters being found from winter to summer on the coasts of Honshu, Shikoku, Kyushu, Loochoo Islands and Korea. In Mutsu Bay it is occasionally abundant during spring and summer. The species is further, though seldom, found on the coasts of Hokkaido and Saghalien as a temporary visitor.

Family CYANEIDAE

Cyanea capillata ESCHSCHOLTZ

Cyanea capillata: UCHIDA, 1927 (a), pp. 230-232.

The medusa is an arctic form and is not rare in summer in waters of Hokkaido and Saghalien, but rather rare in Mutsu Bay. Mainly on the basis of the canal system, species of *Cyanea* seem to be able to be divided into two groups; *capillata*-group and *nozakii*-group. Medusae belonging to the former group are mostly distributed in the boreal regions, while those of the latter are restricted to warm and tropical waters.

RHIZOSTOMAE

Family RHIZOSTOMIDAE

Rhopilema asamushi UCHIDA

Rhopilema esculenta var. *asamushi:* UCHIDA, 1927 (a), pp. 233-236.

In the writer's former report this medusa was described as a variety of *R. esculenta* but it is easily distinguishable from the latter by the brown coloration and more robust appendages of oral arms. This species seems to be widely distributed in the Japan sea, from Mutsu Bay southwards. In medusae used as food material in Korea and Kyushu two forms are found; one brown and the other blue. The blue form is *R. esculenta* and the brown one is probably *R. asamushi*. The writer examined a well-developed specimen of *R. asamushi* which was collected and preserved in the Amakusa Marine Biological Station.

CTENOPHORA

Hormiphora palmata CHUN

Hormiphora palmata: UCHIDA, 1927 (a), p. 236.

This medusa occurs during April-July in Honshu and Hokkaido.

Beroë ramosa KOMAI

Beroë ramosa: UCHIDA, 1927 (a), p. 237.

This ctenophore has been hitherto known only from North Japan.

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MEDUSAE IN ONAGAWA BAY AND ITS VICINITY

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(With seven figures)

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Regarding the medusan fauna of the Northern Pacific coasts of Honshu only very meagre reports have been made. Recently through the kindness of Dr. T. Imai the author had an opportunity to examine the medusae collected by the officers of the Onagawa Oceano-chemical Institute of the Tôhoku Imperial University from Onagawa Bay and its vicinity during these three years. From this collection it has been found that the medusae of Onagawa Bay are mostly in common with those from Mutsu Bay. Influenced by the cold current Oyashio cold water forms, such as *Sarsia tubulosa, Leuckartiara brevicornis, Willsia flavicirrata* and *Eutonia indicans* have been examined in this collection. Carried by the warm current Kuroshiwo, *Pelagia panopyra* was found off Onagawa Bay. As new forms to the Japanese waters *Leuckartiara brevicornis* and *Eucope fragilis* n. sp. have been here enumerated. The synonymic references of most of medusae here considered have mostly been omitted, because they have been given in the report on the medusae from Mutsu Bay (1938).

HYDROMEDUSAE

Anthomedusae

1) *Sarsia tubulosa* (Sars)
2) *Hydrocoryne miurensis* Stechow
3) *Leuckartiara brevicornis* (Murbach et Shearer)
4) *Turritopsis nutricula* Mc Crady
5) *Nemopsis dofleini* Maas
6) *Spirocodon saltatrix* (Tilesius)
7) *Willsia flavicirrata* (Brandt)

Leptomedusae

8) *Eucope fragilis* n. sp.

9) *Eutonia indicans* HARTLAUB
10) *Obelia* sp.
11) *Tima formosa* AGASSIZ
12) *Aequorea coerulescens* (BRANDT)
13) *Gonionemus* sp.

Trachomedusae

14) *Aglantha digitale* MÜLLER

SCYPHOMEDUSAE

Stauromedusae

15) *Haliclystus auricula* CLARK
16) *Sasakiella cruciformis* OKUBO
17) *Sasakiella tsinglaoensis* LING ?

Cubomedusae

18) *Charybdea rastonii* HAACKE

Semaeostomae

19) *Pelagia panopyra* PÉRON et LESUEUR
20) *Dactylometra pacifica* GOETTE

Hydromedusae

ANTHOMEDUSAE

Sarsia tubulosa (SARS)

Sarsia tubulosa : UCHIDA, 1938, pp. 37–38.

Four specimens were collected on June 24, 1936 at Konorihama.

Distribution. North Atlantic coasts of Europe and America. In Japan : Hokkaido and Mutsu Bay.

Hydrocoryne miurensis STECHOW

Hydrocoryne miurensis : UCHIDA, 1938, pp. 37–38.

Four polyps were collected at Ishihama on April 25, 1926. No medusa bud observed.

Distribution. Only in Japan : Pacific coasts of Hokkaido and Honshu, from Akkeshi southwards to Shimoda, Shizuoka Pref.

Leuckartiara brevicornis (MURBACH et SHEARER)

Leuckartiara brevicornis : HARTLAUB. 1913, pp. 304-308; KRAMP, 1926, pp. 80-83; THIEL, 1932, pp. 135-136.

Since the original description this medusa from Alaska by MURBACH and SHEARER, the species has not been recorded from the North Pacific, though HARTLAUB and KRAMP examined fair numbers of this medusa collected in the North Atlantic. This is one of the circumboreal forms. A single specimen was obtained in Okati Bay on May 21, 1937. The specimen measures 20 mm in height and 15 mm in diameter, the apical jelly mass being 6 mm high. Gelatinous substance fairly thick and rigid. Tentacles more than 120 in number, thickly set on the bell margin. They are laterally compressed and rapidly tapering in the distal portion. The tentacle bases are broad but the terminal shafts are narrow and coiled. No ocellus could be seen in this preserved specimen. Radial canals wide and zig-zag on the margins, becoming narrow in the upper portion. Ring canal narrower than the radial canals. Manubrium about 6 mm in length, reaching about midway the bell cavity, with large gastric portion and four well-developed crisped lips.

Fig. 1. *Leuckartiara brevicornis* (MURBACH et SHEARER) ; A side view, B axial view of a part of the bell margin.

Gonads developed on the interradial portions of the manubrium. Each gonad has the typical *Leuckartiara* folds on both sides. The lateral folds are transversely arranged and seven or eight in number. The intermediate portion between these folds presents a complicated meshed appearance ; this feature is quite different from other species of this genus and resembles the gonads of *Neoturris* and some of *Catablema*. The upper part of the gonad is a mixture of folded and meshed structure.

Distribution. Circumboreal, found in both the North Atlantic and the North Pacific.

Turritopsis nutricula Mc CRADY

Turritopsis nutricula: UCHIDA, 1938, p. 40; LING, 1937 pp. 354–355.

About forty examples were obtained at Tori-shima on August 19, 1935.

Distribution. Atlantic coasts of Europe and North America. Mediterranean Sea. In the Pacific, from Hokkaido to Tsingtao. China.

Nemopsis dofleini MAAS

Nemopsis dofleini: UCHIDA, 1927, pp. 223-224; — 1930, p. 334.

About a dozen specimens were collected by a dredge in Onagawa Bay on July 2, 1935 and more than twenty examples were obtained in Koyatori Bay on July 18, 1935. The specimens in these two collections are large, over 20 mm high and about 18 mm wide, but more or less damaged.

Distribution. Only in Japanese waters, from Saghalien, Hokkaido to the Inland Sea, Honshu.

Spirocodon saltatrix (TILESIUS)

Spirocodon saltatrix: UCHIDA, 1938, p. 40.

Four specimens collected in front of the Oceano-chemical Institute on

Fig. 2. *Spirocodon saltatrix* TILESIUS) ×3/5.

Jan. 11, 1936. The smallest specimen is 7 mm high and with 3 long and a pair of short tentacles in each octant. Radial canals give rise to many simple side branches. A large specimen, 62 mm high, 35 mm wide on the apical level and 56 mm wide on the basal level was caught at Konorihama on April 20, 1936.

Distribution. Only in Japan, common in Honshu and Kyushu in February and March.

Willsia flavicirrata (BRANDT)

Willsia pacifica : UCHIDA, 1930, p. 334.
Willsia stellata : UCHIDA, 1930, pp. 334-335.
Willia pacifica : UCHIDA, 1927, p. 236.
Willia stellata : UCHIDA, 1927, pp. 235-236.
Proboscidactylia flavicirrata : BRANDT, J. F., 1838, pp. 390-391.

The following examples were examined ; three collected in front of the Oceano-chemical Institute on Jan. 11, 1936, ten in Onagawa Bay on Oct. 14, 1936 and eight at Konorihama on Oct. 15, 1937. The present author who examined only young specimens of this medusa identified the Japanese medusa with *Willsia stellata*. Recently he collected many examples and has come to the conclusion that the Japanese species is different from the European species in the possession of more tentacles and more exumbrellar nematocyst strings. Moreover the Japanese medusa is variable in the number of groups of radial canals which range from 4 to 9, while the European species has, so far reported, always 6 groups of radial canals. MAAS reported on the Japanese medusa that his specimens has 6 × 18 radial canals and the mouth was complexedly crisped. Among the specimens from Onagawa

Fig. 3. *Willsia flavicirrata* (BRANDT) × 10; six-sided specimen.

Bay there are specimens with 6 × 16 radial canals and 4 × 12 radial canals. In larger specimens the mouth is provided with crisped lips. The further detailed discussion of this species will be published in a paper on the medusae from Akkeshi Bay. The young hexamerous specimen of this medusa cannot be distinguished from the European species, *Willsia stellata*.

LEPTOMEDUSAE

Eucope fragilis n. sp.

Eight specimens were collected on June 21, 1936 at Konorihama.

They are all adult medusae, nearly similar in diameter and in number of tentacles. Body dome-shaped, 9–12 mm high and 5–6 mm wide. Gelatinous substance very thin and soft. Manubrium lacking peduncle, 1–1.5 mm long, four-sided, with well-developed lips. Four radial canals and a ring canal, narrow and straight. Tentacles 16 in all, four perradial, four
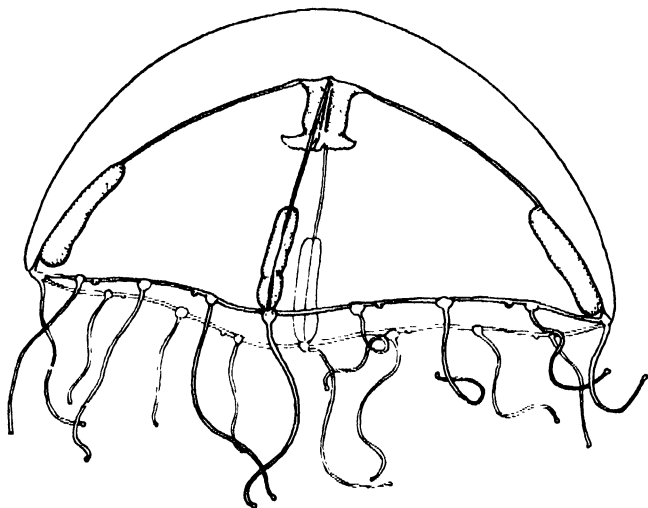
Fig. 4. *Eucope fragilis* n. sp.

interradial and eight adradial, the perradial ones slightly longer than the others and the adradial slightly shorter than the interradial. Tentacles hollow, each composed of a well-developed large tentacle bulb and a narrow distal shaft which is usually coiled in these preserved specimens. Statocysts, eight in number, situated between the adradial and the interradial tentacles, nearer to the former than to the latter. They are mounted on warts arranged along the ring canal. Small warts which are probably rudimentary tentacles are sometimes found midway on the ring canal between the adradial and interradial tentacles. Gonads linear sac-like, developed upon the lower half of the radial canals, shorter than half the length of the radial canals. Colourless in preserved specimens. Manubrium, tentacle bulbs and gonads opaque.

This species is different from most of the other medusae belonging to *Eucope* in having more than eight tentacles. But in regard to the number of tentacles it is probable to be young medusae of *E. globosa* (Haddon) occurring in English and Dutch waters. The Atlantic medusa having 32

or more tentacles in the adult is thick in jelly and higher than a hemisphere in shape. Moreover, gonads of *E. globosa* develop upon the radial canals as four small oval swellings at the middle parts of the four canals, while those of *E. fragilis* n. sp. are oblong and extend near the ring canal.

Eutonia indicans (ROMANCES)

Eutonia indicans: UCHIDA, 1933, p. 131.

A large specimen measuring about 40 mm in diameter was collected on May 11, 1937 at Myozinmae, Okati. Another specimen, smaller than the former, was obtained by Prof. S. HOZAWA at Izushima on April 20, 1931. This species is widely distributed in the North Atlantic.

Distribution in Japan. Kurile Islands and South-Eastern coasts of Hokkaido.

Obelia sp.

Seven specimens were collected on Oct. 20, 1936 in front of the Institute and about a dozen specimens were caught on Oct. 15, 1937 at Konorihama.

Tima formosa AGASSIZ

Tima formosa: UCHIDA, 1925, p. 92.

A single young specimen was collected at Mangoku-ura on Dec. 14, 1936. The bell of the example is dome-like, measuring 34 mm in diameter and 17 mm in height. Peduncle, cylindrical, slightly narrowed distally, hanging below the opening of the bell, 15 mm in the whole length, with manubrium 4 mm in length. Tentacles had mostly been shed off but seemingly about 10 in each quadrant. They are different in length and in this specimen not arranged regularly. The basal bulbs of the tentacles are long and somewhat ribbon-like, while the main shafts of the tentacles are narrow and prehensile. Between the tentacles are found statocysts and rudimentary tentacles, though they are often worn off. Gonads, very narrow, developed on nearly the whole length of the radial canals. They are infrequently waved in the parts of the peduncle but slightly folded in the subumbrellar part, especially in the middle or in the marginal portion. Manubrium with a short gastric part and with four well developed lips which are fairly crisped on the margins. From the slightly developed gonads it seems to be a young specimen of *T. formosa*, which was formerly recorded by the writer from Oshoro, Hokkaido. BIGELOW (1913)

recorded another species *T. saghalinensis* from Saghalien basing on a single large specimen. This species may be a well-developed specimen of the present species but is distinct by the shorter peduncle.

Distribution. American coasts of the North Atlantic. In the Pacific, Northern Japan.

Aequorea coerulescens (BRANDT)

Aequorea coerulescens: UCHIDA, 1938, p. 42.

A large specimen, 95 mm in diameter, was examined. It was caught in June, 1935 at Konorihama.

Distribution. Pacific coasts of Japan. Common in warm waters.

Gonionemus sp.

A damaged specimen. From the form of the umbrella it seems to belong to *G. depressum*. The specimen was obtained on July 17, 1936 in Ommaewan.

TRACHOMEDUSAE

Aglantha digitale MÜLLER

Aglantha digitale: UCHIDA, 1938, p. 43.

Four young specimens were collected off Enoshima on May 19, 1936. This is one of the circumboreal forms. On the Pacific coasts of Japan it was found southwards to Misaki.

Scyphomedusae

STAUROMEDUSAE

Haliclystus auricula CLARK

Haliclystus auricula: UCHIDA, 1938, p. 44.

An adult and two young specimens were collected on August 17, 1935 at Ishihama.

Distribution. In the Pacific from Northern part of Canada through Alaska, Hokkaido, Honshu, Shikoku to Tsingtao.

Sasakiella cruciformis OKUBO

Sasakiella cruciformis: UCHIDA, 1927, pp. 228-229; —, 1929, pp. 146-149; LING, 1937, pp. 16-25; UCHIDA, 1938, p. 44.

Six specimens were collected at Nonohama on August 19, 1935.

These preserved specimens are not so deeply pigmented. Among them the largest specimen is 17 mm in diameter with arms of 2.3 mm long, with eight primary tentacles and in each bunch 17 secondary tentacles.

This species is widely variable in colour. The medusae occurring at Oshoro are all black but those from Mutsu Bay black or brown. According to LING, specimens at Tsingtao are commonly light greenish brown, but variable from light green to deep brown. Moreover, pure green or yellow examples are seldom observed.
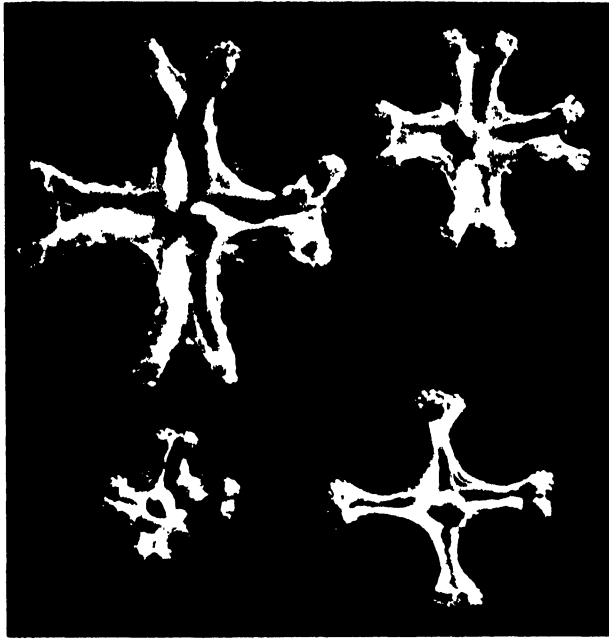
Fig. 5. Aboral views of three specimens of *Sasakiella cruciformis* OKUBO and an example of *Sasakiella tsingtaoensis* LING? which is figured in the lower corner on the right side. × 3.

Distribution. From southern parts of Hokkaido, through Mutsu Bay, to Onagawa. In China, Tsingtao.

Sasakiella tsingtaoensis LING?

Sasakiella tsingtaoensis: LING, 1937, pp. 25 28.

The single specimen caught at Ishihama on August 17, 1935 coincides with the Chinese specimens in the outline of the body The specimen is nearly colourless in preserved condition, 11 mm in diameter and has 15

Fig. 6. Oral views of *Sasakiella cruciformis* OKUBO (on the left) and *Sasakiella tsingtaoensis* LING? (on the right side). ×3.

tentacles in each adradial bunch, which are arranged in two or three rows. Nematocyst clusters sparsely distributed on the exumbrella but richly on the subumbrella. The specimen resembles the Chinese species in the slender body, especially narrowed near the base of the arms and in the lack of the four primary tentacles in the interradii but is quite different from the latter in the lack of the four primary tentacles in the perradii. These features must be noticeable, because *Sasakiella* is distinguished from *Kishinouyea* in the possession of eight primary tentacles in the perradii and interradii. Therefore, the example destitute of all primary tentacles seems to be eligible for *Kishinouyea* and may be identified with *Kishinouyea nagatensis*. The specimen, however, being in general agreement with the Chinese specimen, seems to be an abnormal specimen of the latter.

On account of the occurrence of this specimen and Chinese specimens of *S. tsintaoensis*, however, the distinction between *Sasakiella* and *Kishinouyea* becomes very obscure. Moreover, the coloration of the Chinese *Sasakiella* is like that of medusae belonging to *Kishinouyea*. On the other hand, there exist the two facts that several hundreds of specimens of *S. cruciformis* observed in Japan and China are always provided with eight primary tentacles, while several dozens of individuals belonging to *Kishinouyea nagatensis* always entirely lack primary tentacles. At any rate, *Sasakiella* seems to have the tendency to lose the primary tentacles, first the interradial and then the perradial, and to diverge to *Kishinouyea*. It seems to the writer that it is very important for the study on the interrelation of these two genera to observe the metamorphosis of *Kishinouyea*. The metamorphosis of *S. cruciformis* has been already studied by the writer.

CUBOMEDUSAE

Charybdea rastonii HAACKE

Charybdea rastonii: UCHIDA, 1938, p. 43.

Two young medusae were obtained on November 18, 1936 in front of the Laboratory.

Distribution. In the Pacific, in warm waters. In Japan; from Hokkaido to Formosa.

SEMAEOSTOMAE

Palagia panopyra PÉRON et LESUEUR

Pelagia panopyra: UCHIDA, 1935, p. 43.

Three medusae were collected off Kinkwazan on Sep. 25, 1936. This
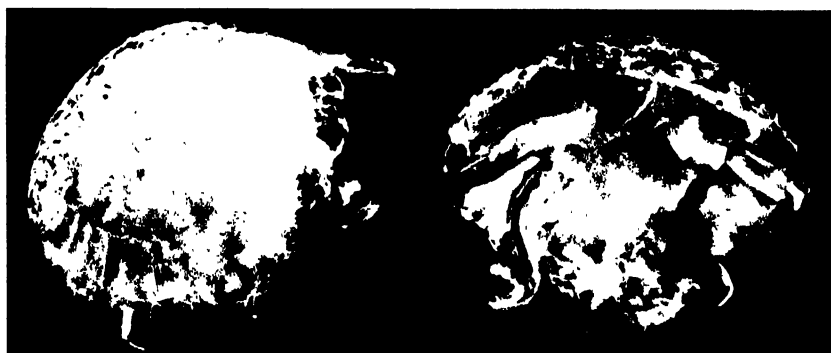
Fig. 7. *Pelagia panopyra* PÉRON et LESUEUR, dorsal and ventral views. ×1.

species is carried on the warm current, Kuroshio. The length of the oral arms is variable in preservation.

Dactylometra pacifica GOETTE

Dactylometra pacifica: UCHIDA, 1935, p. 44.

A specimen was obtained at Konorihama on August 28, 1936.

Distribution. From Honshu to the Loochoo Islands.

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OBSERVATIONS ON THE MARTIN FLY, *STENOPTERYX NIPPONICA* KISHIDA (DIPTERA, HIPPOBOSCIDAE)

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(With Plates II-V)

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I. INTRODUCTION

The louse-flies belonging to the family Hippoboscidae, are very abnormal flies that, in the adult state, live like lice, parasitic upon the bodies of birds and mammals. The external features of these insects are greatly modified so as to adapt them well to their ectoparasitic life, and their life history is also very peculiar. Our knowledge regarding the biology of the Hippoboscidae seems to be rather fragmentary. LEUCKART (1858) and PRATT (1899) studied the embryology and the anatomy of the Sheep Tick, *Melophagus ovinus* and SWINGLE (1913) wrote a paper concerning the biology of the same. ROBERT (1925) gave some accounts of the biology of the Forest Fly, *Hippobosca equina* and discussed many new and interesting facts in regard to these species. HASE (1927), in his study of the Forest Fly, gave notes on its movement and heart beat, and on the process of biting. HARDENBERG (1929) published the results of his elaborate researches on the anatomy, the embryology, and the biology of the Pupipara. He dealt with a number of the Pupipara belonging to the families, Hippoboscidae, Streblidae and Nycteribiidae. In his work he made some interesting contributions in regard to the biology of adults and pupae basing his discussion upon two kinds of bird louse-flies, *Stenopteryx hirundinis* and *Crahterina pallida*. O'ROKE (1930) contributed some notes on the biology of the Quail Fly, *Lynchia hirusta*, and DRAKE and JONES (1930), COATNEY (1931), and others reported on the biology of the Pigeon Fly, *Pseudolynchia maura*.

In fact, as mentioned above, our knowledge concerning the biology of the Hippoboscidae seems to be very scanty and this is true especially in the case of the forms parasitic on wild birds. It is very fortunate for me that I had a good opportunity to study the biology of the Martin

Fly, *Stenopteryx nipponica* at the Tubame Hotspring, Niigata Prefecture, Japan. The Martin Fly was first described by K. KISHIDA in 1931, the specimen being obtained from the body of the Black-chinned Martin, *Delichon urbica dasypus*, commonly found in Japan. Concerning the biology of this fly, no works have been published hitherto. In the present paper I should like to mention the life history of this species, and also to deal with some morphological features, chiefly of the larva of the same.

Before proceeding further, I wish to express my hearty thanks to Professor Dr. SANJI HÔZAWA under whose direction and encouragement my investigations have been made. I am also greatly indebted to MR. KYUKICHI KISHIDA, a member of the Imperial Agricultural Experiment Station, who was very kind in identifying the specimen for me and in giving me many valuable suggestions. I desire also to take this opportunity of thanking MR. HAYAO SASAKAWA, the master of the Hotel Sasaya, Tubame Hotspring, who gave me a great deal of assistance.

II. MATERIAL AND METHODS

The observations were made during the period extending from the beginning of June to the beginning of August at the Tubame Hotspring above mentioned. Tubame Hotspring is situated 1,200 metres above sea-level and is located at the foot of Mt. Myôkô. At Tubame and in its neighbourhood we find a large number of a kind of martin, called commonly the Black-chinned Martin and specifically known as *Delichon urbica dasypus*. The Black-chinned Martin is allied to the European House Martin and is commonly found in Japan. The majority of the Martin Flies were collected from the nests which were built by the martins under the eaves of the buildings belonging to the hotels. The adults and the pupae of this fly were first fixed with either BOUIN's fluid or CARNOY's fluid and then preserved in 80% alcohol. In studying the genital system of the female, both living and preserved specimens were used. To observe the tracheal system of the larva, living materials were chiefly used. The copulation, the deposition of the full-grown larva and various habits of the fly were observed in the glass tubes in which the adult flies were put.

III. THE HOST BIRD

According to my own observations, the host of the Martin Flies seems to be limited to the Black-chinned Martin, as I was not able to find the

same in the nests of other birds such as the Eastern House-swallows, *Hirundo rustica gutturalis*. At Tubame the martins usually build their nests attached to the walls of cliffs or to the walls of caves, but sometimes they build them under the eaves of the buildings of the hotels or of the cottages. In April and May the martin collects pellets of mud or clay and piles them up so as to form a cup-like shelf with its rim coming close against the underside of the eaves. The inside of the nest is lined with such things as straw, feathers, etc. According to the observations of MR. H. SASAKAWA, the martins visit Tubame Hotspring at the end of March and go to the warm countries at the end of September. The breeding season of this bird begins in May and lasts till the end of August. The number of eggs laid for an incubation is three or four.

IV. THE ADULT FLY

The adult fly (Plate II, fig. 1) is greenish-brown in colour, and the entire body is covered with bristly hairs. The female measures from 4.0 to 5.3 mm. in length and the male measures from 4.0 to 4.9 mm. The wings of both sexes exhibit so extensive a reduction that they are apparently useless for the purpose of flight. The female with her abdomen dilated (Plate II, fig. 2) is easily distinguished from the male (Plate II, fig. 3), with penis which may be protruded when the abdomen is pressed gently.

The Martin Flies generally live in the nests of the martins, but some are found attached to the body of the martin. If we destroy the nest, we can observe many Martin Flies escaping from it. The flies by means of their proboscies suck blood from the adult martins and also from their young. On their emergence the young adult breaks the anterior end of the puparium (Plate V, fig. 16) and escapes from the hole thus made. The newly emerged adult has a narrow abdomen and is greenish in colour.

The mating generally takes place while the flies are still in the nest. The male holds the neck of the female with his forelegs and bends his abdomen ventrally to insert the penis. The male moves its abdomen rhythmically during the act of copulation. As in the case of other Hippoboscid flies hitherto known, the egg stage and the larval stage of this kind of fly are completed within the uterus of the mother insect. The gravid female is readily distinguishable by her swollen abdomen. The female deposits the full-grown larva in the nest of the martin. The time spent in the deposition of one larva is only two or three minutes. In the following table is shown the number of the adult flies, together

with their sex ratio, and the number of the puparia found in each nest.

TABLE 1.

*Showing the number of the adult Martin Flies and of the
puparia found in each of 12 nests.*

No. of nest	Date of observation	Number of females	Number of males	Total number of adults	Number of puparia
1	VI 15	4	3	7	12
2	VI 15	6	5	11	14
3	VI 25	4	5	9	7
4	VII 4	7	5	12	15
5	VII 15	4	4	8	13
6	VII 21	13	11	24	16
7	VII 25	6	4	10	47
8	VII 25	12	9	21	16
9	VII 29	7	5	12	18
10	VIII 1	18	12	30	17
11	VIII 1	14	12	26	13
12	VIII 2	11	7	18	21
Total		106	82	188	209

The nests which I observed were all infested with these flies. The maximum number of flies found in one nest was 30, and the minimum was 7. Out of the total of 188 flies found in 12 nests, 106 were female and 82 were male. STEKHOVEN (1926) reported in the case of *Hippobosca maculata* that the sexes were nearly equal in number. HARDENBERG (1929) reported the result of his observations on the sex ratio of some members of the Hippoboscidae, He observed that the sex ratio in the case of *H. rufipes* was almost 1 : 1. In the case of the Sheep Tick the number of the females was much larger than that of the males, that is, out of 840, 501 were female and 339 were male. He considered that the number of the males diminished after their emergence for the two following reasons; first, the life of the male is shorter than that of the female, and second, the male is more active and is more liable to escape from the nest. Regarding the sex ratio of *Stenopteryx hirundinis,* HARDENBERG in searching a number of nests of the House Martins obtained 72 females and 39 males. But the sex ratio of the same fly, when he examined the material newly emerged from the puparia, was almost 1 : 1., that is, out of 42 newly emerged flies, 20 were female and 22 were male. Then he considered that in this case also the males diminished after their emergence. In the case of the present species the number of females is a little larger than that of the males, and I think that this also is due to the reasons given by HARDENBERG.

V. THE GENITAL SYSTEM OF THE FEMALE FLY

The genital system of the female fly occupies, as in the cases of other insects, the ventral position, lying in the posterior portion of the abdomen. Like other Hippoboscid flies hitherto known, the female genital system (Plate II, fig. 4) is made up of the following parts; ovaries, oviduct, a uterus, a vagina, spermathecae, and milk-glands. Each of the paired ovaries (*ov*) appears as an ovoid body of variable size. The peritoneal covering which encloses the ovary is of an extraordinary thickness and is in the form of an elastic sack. Each of the two ovaries consists of two ovarioles, each of these containing two follicles and a germarium. Thus in one ovary there are always found four developing ova, two being contained in each ovariole, and in two ovaries there may exist eight ova. The two ovaries alternately produce a matured egg, and within each ovary the two ovarioles alternately produce an egg. As the entire embryonic and larval growth take place within the uterus, the egg is retained for a long time in the ovary. One of the eight ova contained in the two ovaries is much larger than the others. When the matured ovum is extruded from the ovary, the latter shrinks at once to a very small size and then the ovary of the opposite side increases its size in turn.

The oviduct (*ovi*) may be divided into two parts, that is, two short paired oviducts and a median unpaired oviduct, which is formed by the fusion of the proximal ends of the paired oviducts. In the case of the Sheep Tick, the median unpaired oviduct bears an enlarged portion called the atrium. PRATT (1899) reported that the atrium had the same function as a spermatheca. But in our species the portion to be called the atrium is hardly recognizable.

The uterus (*ut*) is a broad, dorso-ventrally compressed tube stretching from the anterior end of the vagina forward to the median oviduct. The shape and size of the uterus are entirely dependent upon the age and the sexual condition of the female insect, and in the case of a virgin fly the uterus has a length of about 1.3 mm. The matured egg, when extruded from the ovary comes down to the uterus and there it hatches out into a young larva. The presence of an egg in the uterus changes the shape of the uterus slightly, but when the egg hatches and the young larva begins to grow, the uterus rapidly becomes enlarged in accordance with the shape of the larva. The head of the larva staying within the uterus always lies at the anterior end of the uterus.

The milk-gland (*m. gl*) consists of a pair of large, repeatedly branched tubes, fused at their proximal ends so as to form a short unpaired tube. The unpaired tube thus formed and three short tubes forming the spermathecae meet together in one spot and join in a short common tube which pierces the dorsal wall of the uterus at the anterior end and thus pours their secretion into the uterus. The function of these glands may be considered to be to furnish the milk-like fluid serving as food for the growing larva. The milk thus poured into the uterus is sucked by the larva by means of its mouth. The three tubes found near the base of the milk-gland were considered by LEUCKART (1858) and PRATT (1899) as rudimentary milk-glands which have lost their own function. DOUFOUR (1845) stated that these tubes were homologous with the spermathecae, and this opinion was supported by HARDENBERG (1929). I shall also call them spermathecae adopting the opinion of DOUFOUR and HARDENBERG.

VI. THE EGG AND THE LARVA

The matured egg (Plate III, fig. 5) is elongate oval in shape, one end being slightly broader than the other. The length of the egg measures 1.2 mm. and the breadth 0.45 mm. near the middle. The colour of the egg is milky-white and the chorion exhibits a minute net-work. While the egg stays within the uterus, its anterior pole which bears a micropyle is set at the anterior end of the uterus and the posterior pole is directed towards the vagina. In this position the egg hatches into a larva.

The newly-born larva (Plate III, figs. 6 and 7) is a cylindrical maggot of creamy-white with the surface very smooth and glistening. It measures 1.1 mm. in length and 0.50 mm. in breadth. The head is extremely inconspicuous as in the cases of other dipterous larvae of the acephalous type. In the cephalic region there is a pair of lateral lobes. Each of these lobes is divisible into two parts, that is; the basal part and the distal dilated part. Between these two lateral lobes and slightly back of the larval mouth there exists a muscular tongue-like organ (*t. or*), ventral of which the oesophagus passes into the sack-like stomach. Within the uterus of the mother insect the mouth of the larva is always directed towards the opening of the milk-glands. By contraction and dilation of the muscular tongue-like organ, occuring regularly like the heart beat, the larva sucks the milk secreted by the milk-glands. The larva does not show segmentation and thus is immobile. The larva of the first stage

shows but a rudimentary condition of the tracheal system. The tracheal system consists chiefly of a thread-like main trunk passing along each side of the body. Tracheal branches rise dorsally and ventrally from the main trunk. The posterior part of the main trunk is divided into three short branches, each of which protrudes into a papilla projecting from the surface of the posterior part of the body (Plate III, fig. 8).

There are three stages in the larval history. After two moultings the larva becomes full grown and the larval skin of the last stage forms the puparium. The larva of the second stage (Plate III, figs. 9 and 10) is globular in form, the trunk being rounded, and the posterior papillae which were seen in the newly-hatched larva are not recognizable in this case.

The full-grown larva (Plate IV, fig. 11) maintains the same globular form and is milky-white in colour. It measures 3.4 mm. in length and 3.0 mm. in breadth. The cephalic region (Plate IV, figs. 12 and 13) measures 0.2 mm. in breadth and is separated from the trunk by a slight constriction. The tracheal system (Plate IV, fig. 11) is well developed. The tracheal system consists of two main trunks (m. tr) and of two smaller lateral trunks (l. tr), all of these extending almost the whole length of the body and connected with each other by transverse tracheae. Each of the main trunks sends off ten pairs of lateral segmental tracheae. Each of these segmental tracheae is divided into inner and outer branches. The inner segmental trachea (a–j) takes its course inwards and communicates with that of the opposite side on the mid-dorsal line. They arise in the median side of the main trunks and form the dorsal loops bearing small tracheae. The first dorsal connective (a) is rather thin, but the 10th (j) is very thick and is strongly built. The first corresponds to what is called the anterior commissure in some dipterous larvae and the latter corresponds to the posterior commissure in the same. The outer segmental tracheae (I–X) are connected with a thin longitudinal trachea which is called a lateral trunk. The lateral trunk runs along each side of the body taking a somewhat zigzag course. The outer segmental trachea and the lateral trunk send off a number of thin tracheal branches to the main ganglia, the visceral organs and the integument. At the anterior end of the main trunk, several tracheal branches are sent off to the head region. At the posterior end of the body, the larva bears a heavily chitinized plate called the spiracular plate. The spiracular plate is roughly hexagonal in form when it is flattened, and is divisible into two fields. Radiating from the centre of the spiracular plate are six series of spiracular

pores. Each of these pores communicates with each of the small tracheal branches arising from a large spiracular trunk. The three spiracular trunks (*s. tr*) found on each field unite to form a short trachea which joins with the main trunk at a point situated a little below the attachment point of the posterior commissure with the same. The spiracular plate also shows a small central opening (*an*), which is possibly the anus of the larva. When the larva is young, the spiracular plate is milky-white like other parts of the body, but when it becomes older this plate gradually changes its colour from white to grey and finally becomes a shining black. In the cases of a pregnant female carrying a matured larva in her uterus, her abdomen is highly swollen and the black spiracular plate of the larva can be seen through the skin of the adult. When the larva becomes full grown in the uterus of the mother insect, it stops the sucking movement of the mouth and the skin covering the body surface turns stiff. At this time the full-grown larva will be deposited outside leaving the uterus. When the larva is deposited, it is light brown in colour excepting the spiracular plate which is shining black. The integument afterwards becomes chitinized to form the pupal case and also there takes place the gradual darkening of the colour until the puparium at last becomes shining black. According to my observations made on two occasions, the colour of the puparium turned from light brown into black in about two hours after it had been deposited.

In the Sheep Tick, it is reported that the full-grown larva which has been deposited from the mother's uterus turns into pupa after 12 hours. It is also known that in the case of *Hippobosca equina* the full-grown larva pupates within a few hours after the deposition. But in the case of our fly, it is somewhat different, that is, when the larva is deposited, it is light brown in colour and the larval skin is rather thickly chitinized. Such a stage may be looked upon as a transitional stage in which the larva turns into the pupa.

VII. THE PUPA

The puparium (Plate V, figs. 14 and 16) is oval in shape and is slightly flattened dorso-ventrally. It measures 2.8 mm. in length and 2.3 mm. in breadth. It is shining black in colour and is without any trace of segmentation, but when it is observed under the microscope a structure of fine net-work can be seen. The number of the puparia found in each nest of the 12 cases investigated is shown in Table 1. The

maximum number found in one nest was 47. As the whole developmental course of the pupa is hidden inside the old larval skin, the puparium, it is necessary to remove the puparium in order to observe the pupa. The pupal body (Plate V, fig. 17) is very soft and white in colour. The proboscis sheath is thick and large, its extreme end reaching to the middle of the body. The three thoracic segments are still distinct, and the appendages are relatively thicker than those of the adult and are placed closely against the ventral surface of the body. The wing sheaths lie flat against the latero-ventral surface of the body, and within these the folded wings can be seen. The third pair of legs extends downwards reaching to the end of the body.

The pupal period, which I observed on one occasion, covered 23 days. When I visited Tubame Hotspring on the 17th of October, the martins had gone to the southern countries and thus no Martin Flies were seen in the nests, but I found some puparia in them. The pupal body which was found within the puparium was not yet developed. The young adults will emerge from their puparia in the spring of next year when the martins came back to their old nests again.

In his observations made on *Stenopteryx hirundinis* which lived in the nest of the European House Martin, HARDENBERG (1929) found some puparia in the nest in winter also. He observed the fact that from the puparia deposited in summer the adults usually emerged after 22 days, and that from the puparia deposited in autumn the adults emerged in the spring of the next year, the pupal stage being about 6 months. HARDEN-BERG called the pupa which was deposited in summer „ Sommerpuppe ", and the same which was deposited in autumn „ Winterpuppe ". In the case of our flies also the distinction between the summer pupa and the winter pupa is recognizable.

VIII. SUMMARY

1) The Martin Fly, *Stenopteryx nipponica* KISHIDA infests the Black-chinned Martin, *Delichon urbica dasypus* and is found in its nest.

2) All the nests which I observed were infested with the Martin Flies. The number of flies found in each nest ranged from 7 to 30.

3) The female genital system of the Martin Fly consists of a pair of ovaries, paired oviducts and a median unpaired oviduct, a uterus, a vagina, a pair of milk-glands, and three spermathecae.

4) The egg is elongate oval in shape and measures 1.2 mm. in length

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and 0.45 mm. in breadth. The egg hatches out in the uterus of the mother insect.

5) The newly-born larva is milky-white in colour and is elongate oval in shape. By contraction and dilation of a tongue-like organ, the larva sucks the milk secreted by the milk-glands of the mother insect. The larval stage consists of three instars. The full-grown larva is oval in shape and measures 3.4 mm. in length and 3.0 mm. in breadth. The posterior abdominal spiracular plate of the full-grown larva is highly chitinized and shining black in colour.

6) The full-grown larva covered with more or less chitinized skin is deposited in the martin's nest by the mother insect.

7) The puparium is oval in form and shining black in colour. It measures 2.8 mm. in length and 2.4 mm. in breadth. The pupa which has been deposited during the period covering the end of the summer and the beginning of the autumn hibernates during the winter and the adult emerges in the next spring.

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EXPLANATION OF THE PLATES

Stenopteryx nipponica KISHIDA

PLATE II

Fig. 1. A gravid female. (×10)
Fig. 2. Abdomen of the female seen from the ventral side. (×15)
Fig. 3. Abdomen of the male seen from the ventral side. (×15)
Fig. 4. Female genital system.
lar larva, m. gl milk-gland, ov ovary, ovi oviduct, sp spermatheca, ut uterus, vag vagina.

PLATE III

Fig. 5. An egg. (×65)
Fig. 6. A newly-born larva seen from the right side. (×65)
Fig. 7. The same seen from the ventral side. t.or tongue-like organ. (×65)
Fig. 8. Posterior part of the newly-born larva. (×200)
Fig. 9. The larva of the second stage seen from the ventral side. (×50)
Fig. 10. Head region of the same seen from the ventral side. (×200)

PLATE IV

Fig. 11. Tracheal system of the full-grown larva. (×40)
a–j inner branches, I–X outer branches, m.tr main trunk, l.tr lateral trunk, s.tr spiracular trunk, an anus.
Fig. 12. Head region of the full-grown larva seen from the dorsal side. (×200) t.or tongue-like organ.
Fig. 13. The same seen from the ventral side. (×200)

PLATE V

Fig. 14. Puparia and their exuviae. (×ca. 1.5)
Fig. 15. Spiracular plate of the puparium. (×30)
Fig. 16. Puparium. (×ca. 20)
Fig. 17. Pupa. (×ca. 20)
Fig. 18. Exuviae of the puparium. (×ca. 20)

K. HUZIMATU: Observations on Martin Fly.

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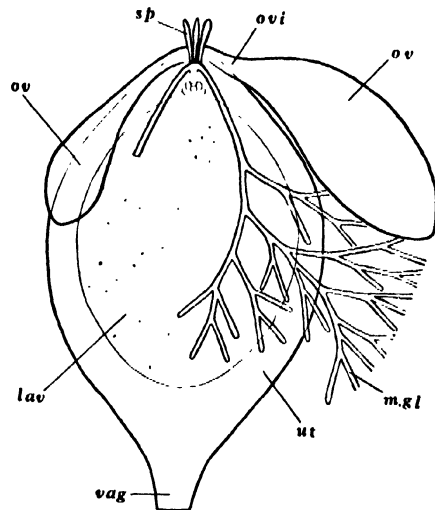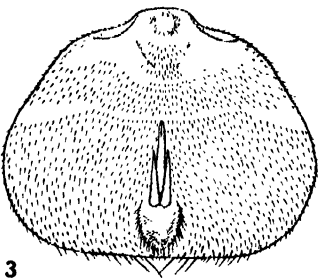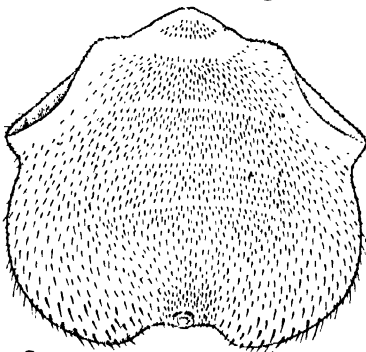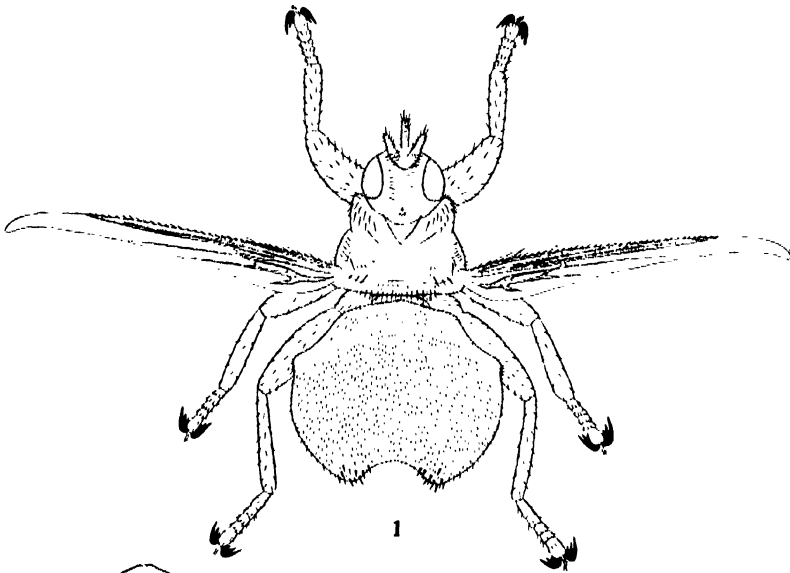
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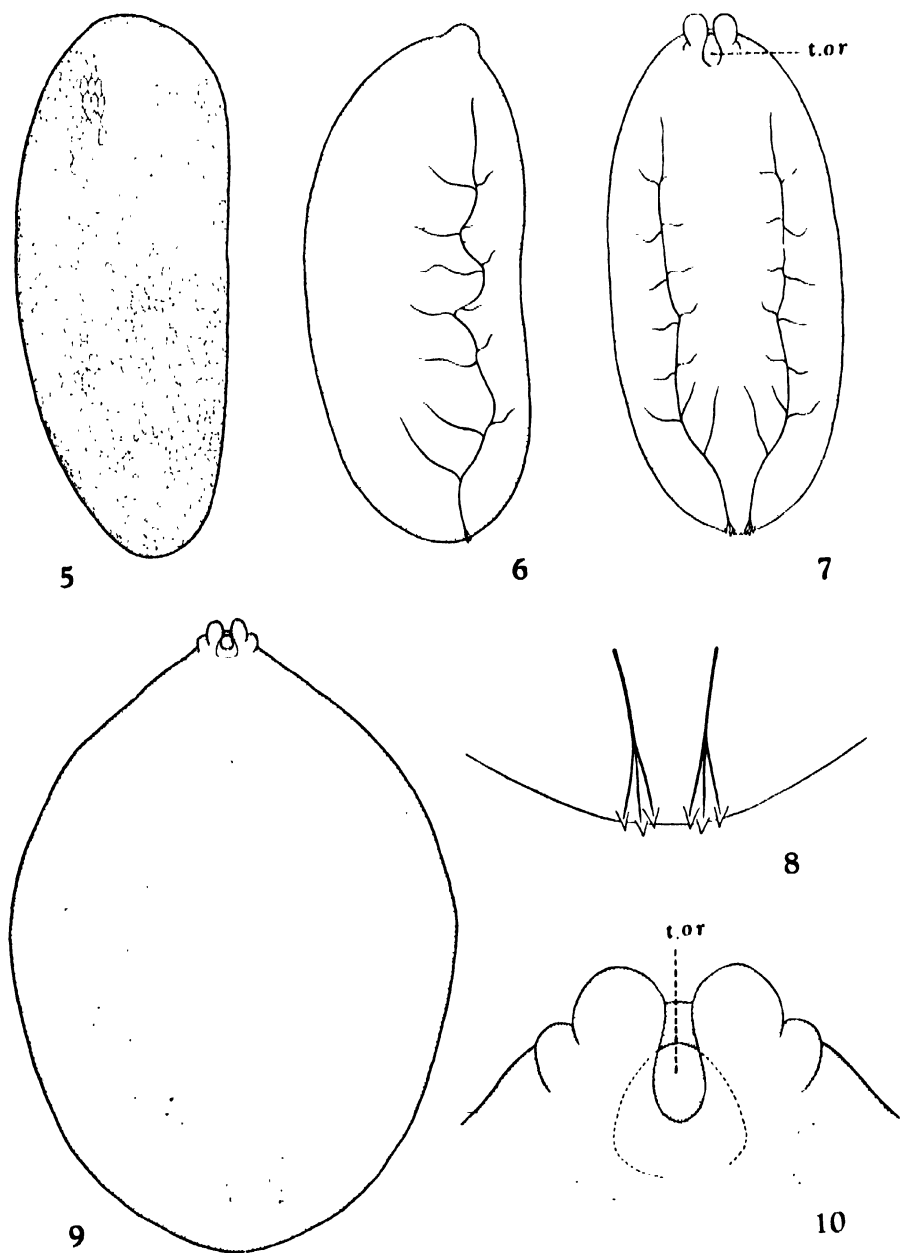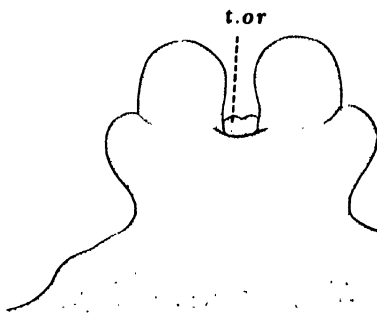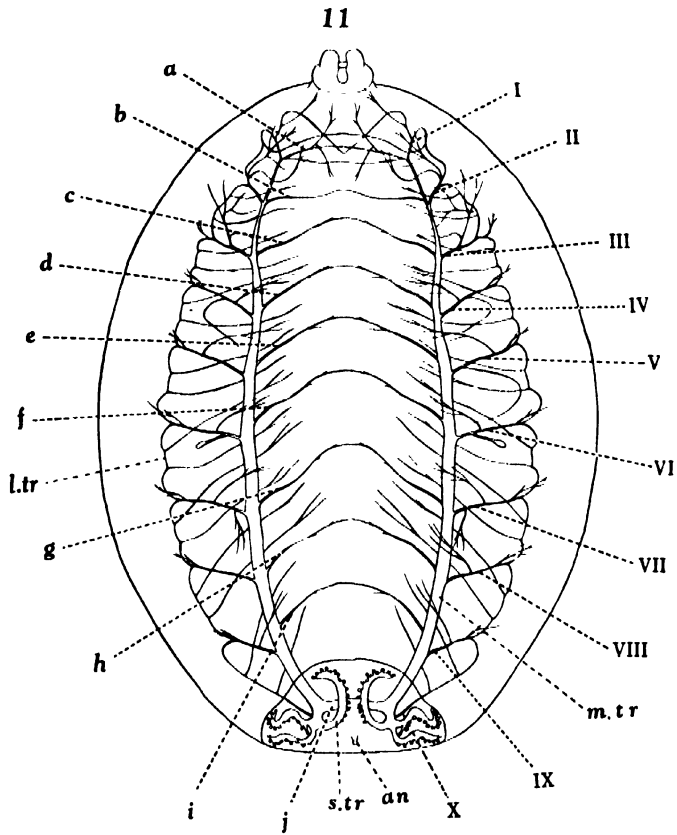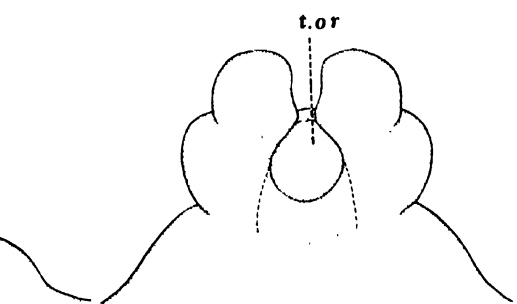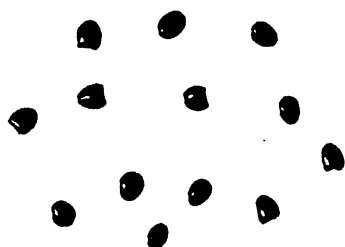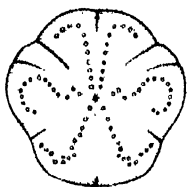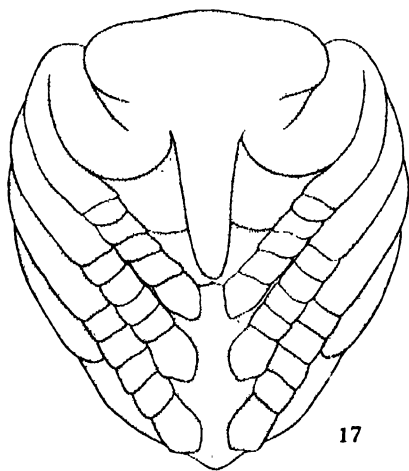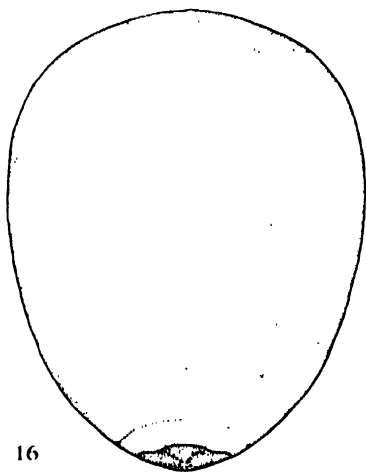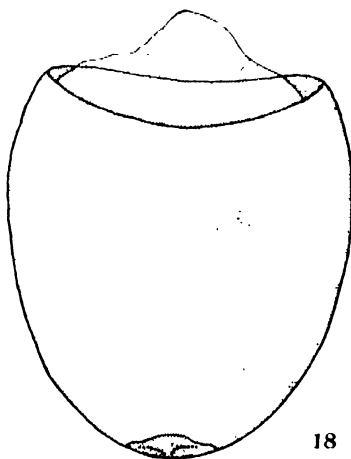
SYMBOLAE ITEOLOGICAE V

AUCTORE

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(Cum 4 tabulis et 6 figuris in textu)

(Opus acceptum d. XX m. Maji a. MCMXXXVIII)

42) × **Salix Ikenoana**[1] KIMURA hyb. nov. (Fig. 1 & Tab. VI, VII).

= *Salix integra* THUNBERG × *S. sachalinensis* SCHMIDT.

Descr. specim. original.: *Frutex* ramulosus 2 m altus. *Ramuli* graciles viminales adscendentes glabri, hornotini aestate virides, annotini gemmiferi paullo nitentes, latere soli aperto purpurascentes, altero abscondito brunneo-virides, biennes cinereo-viriduli ; *rami* viridi-cinerei vel cinerei. *Gemmae* in ramulis condensatae alternae superne solutae, amentiferae oblongae vel elliptico-oblongae apice obtusissimae, latere paullo vel vix carinatae, ventre moderatim convexae sub lente pulverulento-puberulae, dorso glaberrimae valde convexae, purpureo-rubrae nitentes, 6–7 mm longae circiter 2.5 mm latae; foliiferae sat minores anguste ovatae apice obtusissimae amentiferis concolores fere glabrae vel minutissime puberulae circiter 3 mm longae 1.5 mm latae. *Cataphylla* sterilium ramulorum viridia, prima et secunda lateralia, sessilia, elliptico-oblonga ad apicem basimque obtusa, margine integerrima, supra glaberrima infra secus costam adpresse pilosa, 7.5–10.3 mm longa 3–4.5 mm lata, 2.2–2.5-plo longiora quam latiora. *Folia recentissima* utrinque minutissime pubescentia, e vernatione relaxata margine infero revoluta ad modum *S. sachalinensis*. *Folia adulta* chartacea internodiis 6–15 mm longis dissita, *inferiora* oblongo-lanceolata vel elliptico-oblonga, apice acuta ad obtusa basi obtusissima, margine obsolete crenata vel fere integra, supra viridia infra pallidiora, utrinque glaberrima, 4–6 cm longa 1.5–1.7 cm lata, *superiora* lanceolata vel oblongo-lanceolata medio fere latiora, apice acuminata basi obtusa vel obtusissima, 8–11.7 cm longa 1.4–2.0 cm lata, 4.7–5.8-plo longiora quam latiora, margine crenato-serrulata, serraturis in medio folii 4–5 pro 1 cm sursum crebris, basi integra, supra sat viridia non stomatifera leviter tenuiterque impressi-nervata, subtus

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[1] Nomen hoc dedi in honorem Doctoris S. IKENO, Botanicae Professoris Honorarii Imperialis Universitatis Tokyensis, qui primus hanc hybridam aliasque multas japonicarum Salicum arte fecit.

glaucina, utrinque glaberrima ; costa pallida supra elevata infra prominente, utrinque glaberrima ; nervis primariis tenuibus leviter arcuatis supra paullum impressis infra acute elevatis, utroque latere 22–24, sub angulis 60°–70° e costa orientibus, intermediis 1–3, secundariis tenuissimis infra leviter elevatis inter primarios fere transversis. *Petioli* supra sulcati pulchre rubri basi sub lente ferrugineo-puberuli, infra virides nitentes glabri 5–6 mm longi. *Stipulae* oblique ovatae, apice acuminatae, margine minute serrulatae, infra glaucinae, utrinque glaberrimae vel infra solum ex parte puberulae, 5–6.5 mm longae 1.7–2 mm latae. *Amenta* ☿ praecocia in ramulis condensata oblongo-cylindrica densiflora sessilia, rhachidibus pubescentibus, sub anthesi 2–2.8 cm longa 8–9 mm crassa, basi cataphyllis 3–4 oblongis utrinque obtusis margine integerrimis supra fere glabris infra adpresse albosericeis (4.2–) 6–9 mm longis 1.7–3 mm latis, 2.4–3.5-plo longioribus quam latioribus suffulta. *Bracteolae* obovato-oblongae apice obtusae vel obtusissimae, intus concavae extus convexae, utrinque albo-villosae, basi pallide. virides medio brunneae antice nigrae, in

Fig. 1. *Salix Ikenoana* KI-MURA. *A* Flos ☿. *B* Flos ☿. *C* Basis filamentorum. *D*. Antherae a facie.

expansione 1.8–2.0 mm longae 0.8 mm latae. *Glandula* una ventralis oblonga apice truncata viridis 0.7–0.9 mm longa vix 0.3 mm lata. *Stamina* 2, filamentis pallidis 4.0–5.5 mm longis basi paucipilosis, e basi ad 1/4, 1/3, 1/2, 3/4 totae longitudinis connatis. *Antherae* ovales ante explicationem rubrae, effuso polline fuscescentes, circiter 0.6 mm longae.

Nom. Jap. *Ikenoyanagi* nom. nov.

Hab. in Japonia. Honsyû.— Prov. **Rikuzen**: Sendai, (A. KIMURA n. 2350 ☿ fl. [typus] 15 Apr. 1937 in Herb. A. KIMURA, fol. [typus fol.] 13 Oct. 1937, fol. 6 Oct. 1934, fl. 13 Apr. 1935, fol. 17 Sept. 1935, fl. 21 Apr. 1936, gemmif. 22 Feb. 1937, fol. 21 Jun. 1937).— Prov. **Sinano**: Kirigamine, (H. TOBITA st. 22 Jul. 1932).— Prov. **Sagami**: Atuki, (Z. MATUNO st. 23 Jun. 1935).— Prov. **Rikutyû**: Kokarumaimura, (G. TOBA st. 4 Aug. 1935).

Hanc formam prolem esse hybridam inter *S. integram* et *S. sachalinensem* omnes ejus demonstrant partes; solitarie crescit in societate parentum in humidis et ad fluviorum ripas. Ad illam vergens ob habitum

fruticosum, texturam et serraturam foliorum, antheras toto rubras, filamenta basi pilosa et alte connata. A *S.* autem *sachalinensi* habet figuram et dispositionem gemmarum, folia juvenilia margine infero revoluta, adulta apice acuminata supra impresse subtusque elevate nervata, nervos tertios regulares, stipulas vulgo evolutas nec non figuram coloremque bracteolarum. Glandulae forma et magnitudo inter parentes fere mediae. — Optime congruit cum hybrida[1] ab ill. Dr. S. IKENO ex parentibus supra memoratis arte facta, quamquam flores abs qua nondum vidi.

43) **Salix futura** SEEMEN, Salic. Jap. p. 71, t. 17, fig. F G[1] (1903). SCHNEIDER in SARGENT, Pl. Wilson. III. p. 177 (1916). MATSUMURA, Ind. Pl. Jap. II. 2, p. 9 (1912). MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1123 (1925); ed. 2, p. 164 (1931). NEMOTO, Fl. Jap. Suppl. p. 107 (1936). KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. XI. p. 244 (1936).

var. **rufa** KIMURA var. nov. (Fig. 2).

A typo recedit indumento amentorum cataphyllorum foliorumque adultorum rufescente.

Descr. specim. originalis: *Ramuli* in sicco fusci, hornotini pilis ferrugineis cum cinereis mixtis pubescentes, demum glabrescentes, annotini glabri. *Folia adulta* chartacea elliptico-oblonga vel elliptica, apice acuminata, basi obtusissima ad rotundata, margine anguste reflexa, grosse crenato-serrata, dentibus glandulosis in medio folii 3 8 mm inter se remotis, 11–13.5 cm longa, 4.1–5.3 cm lata, supra viridia praeter nervos glabra, infra dilute glauca pilis brevibus ferrugineis

Fig. 2. *Salix futura* SEEMEN var. *rufa* KIMURA. Typus.

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[1] Confer IKENO, On Hybridisation of Some Species of Salix. Jour. Gen. VIII. p. 58 (1918). In hoc opere appellantur "*S. purpurea multinervis*" pro "*S. integra*" et "*S. opaca*" pro "*S. sachalinensis*".

cum albis mixtis obsita ; costa supra plana pilis cinereis minutissimis pulverulento-pubescente, infra vehementer prominente pilis ferrugineis cum albis paucis intermixtis sericea ; nervis primariis arcuato-ascendentibus, utrinque 7--10, a costa sub angulis 55°-70° divergentibus, supra fere planis (saltem in sicco) minutissime pulverulento-pubescentibus, infra prominentibus indumento ut in còsta obsitis, secundariis supra glabris, infra elevatis inter primarios subparallele transversis minute ferrugineo-albo-pilosis. *Petioli* semiteretes supra ad basin sulcati, sordide cinereo-ferrugineo-pubescentes, ad 1.4 cm longi. *Stipulae* bene evolutae oblique ovatae, apice acutae, margine crenato-serratae, supra in nervis cinereo-pubescentes, infra dilute glaucinae, ferrugineo-pubescentes, 8 12 × 5-6 mm etc. magnae. *Amenta* ♀ praecocia longe cylindrica densiflora sessilia, sub anthesi 2.5- 3.3 cm longa 5 mm crassa, basi cataphyllis 3- 5 ovato-ellipticis apice obtusis vel acutis basi sessilibus supra glabris infra pilis ferrugineis cum albis mixtis villosis, 6-7 mm longis 2.5 3.5 mm latis suffulta. *Bracteolae* ovatae apice obtusissimae 1.2 mm longae 0.8 mm latae, in sicco superne nigrescentes inferne brunneae, utrinque pilis ferrugineis cum albis mixtis 1.0- 1.5 mm longis villosae. *Glandula* una ventralis ovata vel ovato-oblonga, apice truncata, 0.7 0.8 mm longa 0.4 0.6 mm lata. *Ovaria* ex ovata basi longe conica, sericea, 1.5-2 mm longa 0.8 mm crassa, subsessilia ; stylis obcompressis 0.8 0.9 mm longis, apice bifidis laciniis divaricatis 0.2-0.3 mm longis. Stigmata parva emarginata.

Hab. in Japonia. Honsyû. Prov. **Etizen** : Simeimura-Mizuoti. (Y. HORI n. 23 fol. adult. [typus var.] 28 Aug. 1937 in Herb. A. KIMURA, ♀ fl. 6 Apr. 1937 ; n. 24 ♀ fl. 6 Apr., fol. 28 Aug. 1937).— Prov. **Ettyû** : sine loco speciali. (T. OTAYA ♀ fl. Apr. 1931).

44) **Salix rupifraga** KOIDZUMI in Tokyo Bot. Mag. XXXIII. p. 121 (1919).— MATSUMURA, Shokubutsu-Mei-I, ed. 10, II. p. 597 (1922).— MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1128 (1925) ; ed. 2, p. 171 (1931). — NEMOTO, Fl. Jap. Suppl. p. 114 (1936).

Fig. 3 & Tab. VIII, IX.- Descr. ♂ : *Frutex* breviter ramulosus ; *ramulis* hornotinis in sicco fuscis sericeo-pubescentibus, annotinis divaricatis glabris vel minute pubescentibus cinereo-fuscis cicatricosis, 2 11 cm longis, vetustioribus cinereo-fuscis rugulosis. *Cataphylla* prima sterilium ramulorum ventralia, ovalia ad ovato-elliptica, apice obtusissima basi leviter cordata, obsolete serrulata, supra glabra subtus adpresse denseque albovillosa, 6-9 mm longa 4.5-5.5 mm lata, secunda dorsali-lateralia anguste ovata apice acuta basi rotundata, 10-14 mm longa 5-6 mm lata, ceterum

ut prima. *Folia recentissima* sub vernatione convoluta utrinque villoso-sericea; *adulta* internodiis 1–2 cm longis dissita, lanceolato-elliptica vel elliptico-oblonga, apice acuminata basi obtusissima vel subrotundata, margine remote mucronulato-serrata, serraturis in medio folii 3–5 pro 1 cm, supra viridia praeter costam paullo impressam minute puberulam fere glabra, subtus glauca adpresse albo-villoso-sericea, 4 6-6.2 cm longa 1.7 2.0 cm lata, 2.7 3.0-plo longiora quam latiora, costa infra prominente adpresse villosa, nervis primariis infra elevatis utrinque 6–9 arcuatis a costa sub angulis 40°–60° divergentibus, secundariis vix elevatis inter primarios transversis subregularibus, intermediis 1–2. *Folia inferiora* elliptica apice obtuse acuta, basi subrotunda superioribus minora. *Petioli* pubescentes supra canaliculati ad 6 mm longi. *Stipulae* nullae. *Amenta* ♂ coaetanea longe cylindrica, versus apicem

Fig. 3. *Salix rupifraga* KOIDZUMI. *A* Flos ♂ a ventre. *B* Idem a latere. *C* Antherae a facie.

angustata, densiflora, 1.8 2.6 cm longa, 6 mm crassa, rhachidibus pubescentibus, pedunculis ad 4 mm longis albo-villosis; cataphylla pedunculorum plerumque 2, iis sterilium ramulorum simillima. *Bracteolae* flavescentes concolores ellipticae ad ovales, apice obtusissimae, intus concavae extus convexae, utrinque albo-villosae, in expansione circiter 1.3 mm longae 0.7 mm latae. *Glandula* una ventralis ovata apice obtusissima 0.5–0.6 mm longa 0.3 0.35 mm lata. *Stamina* 2, filamentis liberis glabris 2 2.5 mm longis. Antherae late ovales luteae 0.6 mm longae.

Nom. Jap. *Iwayanagi* (non T. IWASAKI nec K. ITO nec J. MATSU-MURA) KOIDZUMI in Tokyo Bot. Mag. XXXIII. p. 121 (1919). *Koma-iwayanagi* KOIDZUMI in sched. Herb. Univ. Imp. Kyotensis.

Hab. in Japonia. Honsyû — Prov. **Kai**: in rupium fissuris, (S. HUNO ♂ fl. [typus ♂] 26 Apr. 1936 in Herb. A. KIMURA, fol. 7 Jun. 1936). Folia et flores ex eadem stirpe!

var. **eriocarpa** KIMURA var. nov. (Fig. 4).

A typo ovariis sericeis dignoscenda.— *Ramuli* hornotini albo-sericei basi albo-villosi, annotini fusci rugulosi 2.5–11 cm longi. *Cataphylla* prima sterilium ramulorum adaxialia ovata, apice obtusa basi subrotunda, margine

obsolete serrulata, supra glabra infra dense adpresseque albo-villosa, 1.0–
1.3 cm longa 0.5–0.9 cm lata, secunda ovato-elliptica vel elliptica apice
obtuse acuta basi obtusa ad subrotunda, margine serrulata, supra et infra
ut in primis, 1.7–2.5 cm longa 0.7–1.1 cm lata. *Folia recentissima* sub
vernatione convoluta utrinque adpresse albo-villosissima ; *adulta* oblongo-
elliptica ad vel paullo supra medium latiora, apice subproducto-acuminata
basi obtusissima ad rotundata, margine praeter apicem integerrimam den-
tato-serrata, serraturis in
medio folii 3–4 pro 1 cm et
basi obsoletis, supra villis
adpressis conspersa, subtus
glauca adpresse albo-villoso-
sericea, 5.5–7 cm longa 2–
2.5 cm lata ; costa supra
minute albo-pubescente, sub-
tus prominente adpresse
albo-villosa, nervis primariis
infra parum elevatis utrinque
9–10 leviter arcuatis sub
angulis 40°–50° a costa ori-
entibus, secundariis infra vix
elevatis subregularibus cum
tertiis anastomosantibus.
Petioli villosi supra sulcati
ad 0.8 cm longi. *Stipulae*
nullae. *Amenta* fructifera
tantum visa ad 7 cm longa
circiter 0.8 cm crassa, rhachi-
dibus pubescentibus, pedun-
culis ad 3 mm longis villosis.
Cataphylla pedunculorum 2–

Fig. 4. *Salix rupifraga* KOIDZUMI
var. *eriocarpa* KIMURA. Typus.

3, iis sterilium ramulorum paullo angustiora, prima 0.9–1.1 cm longa 0.5 cm
lata, secunda ad 1.7 cm longa 0.7 cm lata, ceterum ut in sterilium ra-
mulorum. Flores ♀ inter fructus remanentes : *Bracteolae* late ellipticae
apice obtusissimae, flavescentes concolores utrinque albo-villosae, 0.6–1.0 mm
longae 0.6–0.7 mm latae. *Glandula* una ventralis anguste ovata vel oblonga.
apice obtusissima vel fere truncata, 0.7–0.8 mm longa 0.35–0.4 mm lata.
Ovaria ovato-ellipsoidalia sericea circiter 1.6 mm (in fructu ad 3.5 mm)
longa 0.7 mm crassa ; stipitibus 0.4 mm (in fructu 0.6 mm) longis pilosis ;

stylis circiter 0.5 mm longis obcompressis. *Stigmata* parva bifida.

Hab. in Japonia. Honsyû.— Prov. **Kai**: in rupium fissuris, Komaki-mura; (S. HUNO [typus var.] 7 Jun. 1936 in Herb. A. KIMURA); inter saxa, ad ripas fl. Oziragawa, Sugawaramura, (S. HUNO n. 40 fructif. 4 Jun. 1937).

45) **Salix chaenomeloides**[1] KIMURA nom. nov.

Syn. *Salix subfragilis* " ANDERSSON " MATSUMURA in Tokyo Bot. Mag. VIII. p. 152 (1894) **Syn. nov.**

Salix glandulosa (non RAFINESQUE) SEEMEN in ENGLER, Bot. Jahrb. XXI. Beibl. LIII. p. 55 (1896); ibid. XXIX. p. 276 (1900) (DIELS, Fl. Central-China); Salic. Jap. p. 22, t. I, fig. A–F (1903).— SHIRAI in Tokyo Bot. Mag. XVII. p. 223, t. IV (1903).— LÉVEILLÉ in Bull. Acad. Intern. Géogr. Bot. XIV. p. 208 (1904).— GÄRTNER, Vergl. Blattanat. zur Systematik der Gattung Salix p. 26 (1907).— PAVOLINI in Nuov. Giorn. Bot. Ital. n. ser. XV. p. 439 (1908).— NAKAI in Jour. Coll. Sci. Tokyo Imp. Univ. XXXI. p. 214 (1911) (Fl. Korea. II.); Veg. Chirisan Mts. p. 28, no. 105 (1915); in Bull. Soc. Dendr. France no. 66, p. 7 (1928); Fl. Syl. Korea. XVIII. p. 77 (1930).— MATSUMURA, Ind. Pl. Jap. II. 2, p. 10 (1912); Shokubutsu-Mei-I, ed. 9, p. 352 (1916).— KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 87 (1913).— MAKINO & NEMOTO, Cat. Jap. Pl. Herb. Nat. Hist. Dept. Tokyo Imp. Mus. p. 309 (1914); Fl. Jap. ed. 1, p. 1123 (1925); ibid. ed. 2, p. 164 (1931).— SCHNEIDER in SARGENT, Pl. Wilson. III. p. 98 (1916); in BAILEY, Gentes Herb. 1. p 17 (1920). - MORI, Enum. Pl. Corea p. 109 (1922).— REHDER in Jour. Arnold Arb. IV. p. 138 (1932).— KIMURA in Tokyo Bot. Mag. XLII. p. 69 (1928); in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. XI. p. 250 (1936) excl. var.— GÖRZ, Schedae ad fasc. I. Salicac. Asiatic. p. 5 (1931).— NEMOTO, Fl. Jap. Suppl. p. 107 (1936).

Salix triandra var. *vulgaris* " WIMMER " SEEMEN in ENGLER, Bot. Jahrb. XXIX. p. 276 (1900) fide SCHNEIDER.

Salix cardiophylla " TRAUTVETTER & MEYER " WILSON in Jour. Arnold Arb. I. p. 36 (1919) fide NAKAI.

Salix glandulosa SEEMEN var. *glabra* NAKAI, Fl. Syl. Korea. XVIII. p. 80 (1930).

Nom. Jap. *Marubayanagi* [SAITO, Honzo-Kagami, annis *Kyôho* (1716–1735) MS.— IINUMA, Sômoku-Zusetu, Pars II. Vol. IX. anno 3. *Tenpo*

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[1] Epitheton datum est propter stipulas magnas, quae memoriam quorundam specierum Rosacearum generis *Chaenomelis* vivide revocant.

(1832) MS] MATSUMURA in Tokyo Bot. Mag. VIII. p. 152 (1894).— *Aka-ziku-Marubayanagi* [SAITO, Honzo-Kagami, annis *Kyôho* (1716–1735) MS] SHIRAI in Tokyo Bot. Mag. XVII. p. 223 (1903).— *Uraziroyanagi* [IINUMA, Somoku-Zusetu, Pars II. Vol. IX. anno 3. *Tenpo* (1832) MS] SHIRAI in Tokyo Bot. Mag. XVII. p. 223 (1903).— *Akameyanagi* [MATSUMURA in sched. ex Herb. Univ. Imp. Tokyensis] SEEMEN, Salic. Jap. p. 23 (1903).

Hab. in Japonia (Honsyû, Sikoku, Kyûsyû), Korea, Sina centrali.

var. **pilosa** (NAKAI) KIMURA comb. nov.

Syn. *Salix glandulosa* var. *pilosa* NAKAI, Fl. Syl. Korea. XVIII. p. 79 (1930).

Hab. in Korea.— Haec varietas imprimis Koream habitare videtur.

46) **Salix Wilsoni** SEEMEN in ENGLER, Bot. Jahrb. XXXVI. Beibl. LXXXII. p. 28 (1905).— LÉVEILLÉ in Bull. Soc. Bot. France, LVI. p. 301 (1909) ; in Mem. R. Acad. Ci. Art. Barcelona, ser. 3, XII. no. 22, p. 21 (1916) (Cat. Pl. Kiang-Sou).— SCHNEIDER in SARGENT, Pl. Wilson. III. p. 40 (1916) ut *Wilsonii.*— REHDER in Jour. Arnold Arb. VIII. p. 93 (1927) ; ibid. X. p. 112 (1929) ; ibid. XVIII. p. 254 (1937) ut *Wilsonii.*— HANDEL-MAZZETTI, Symb. Sin. VII. p. 60 (1929) ut *Wilsonii.*- - W. C. CHENG in Contr. Biol. Lab. Sci. Soc. China, bot. ser. IX. no. 1, p. 61 (1933) (Enum. Vascular Pl. Chekiang II.).--- SHUN-CHING LEE, Forest Botany of China p. 186 (1935) ut *Wilsonii.*· K. S. HAO in FEDDE, Rep. Sp. Nov. Reg. Veg. Beih. XCIII. p. 41, pl. 1, fig. 2 (1936) (Synop. Chinese Salix).

Syn. *Salix Mesnyi* BURKILL in Jour. Linn. Soc. XXVI. p. 530 (1899) p. p. non HANCE.

Salix Argyi LÉVEILLÉ in FEDDE, Rep. Sp. Nov. Reg. Veg. IX. p. 473 (1912).

Salix glandulosa var. *Wilsonii* GÖRZ in·FEDDE, Rep. Sp. Nov. Reg. Veg. XXXVI. p. 21 (1934).— KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. XI. 2, p. 250 (1936) (Symbol. Iteolog. II.).

Est sine dubio *S. chaenomeloidi* KIMURA ita affinis, ut GÖRZ ill. in ejus varietatem reduxerit ; vetant tamen folia adulta minora, glandulae petioli vulgo non evolutae nequaquam phylloideae, stipulae deficientes vel parum evolutae.— Vidi multa specimina in herbariis Arboreti Arnoldiani et Horti Botanici Noveboracensis conservata.

47) **Salix Rosthornii** SEEMEN apud DIELS in ENGLER, Bot. Jahrb. XXIX. p. 276, t. II. fig. E–H (1900) (DIELS, Fl. Central-China).— ? LÉVEILLÉ in

Bull. Soc. Bot. France, LVI. p. 300 (1909).— SCHNEIDER in SARGENT, Pl. Wilson. III. p. 40 (1916). - HANDEL-MAZZETTI, Symb. Sin. VII. p. 60 (1929).— SHUN-CHING LEE, Forest Bot. China p. 213 (1935).

Gemmae glaberrimae, perulis ventre liberis et imbricatis. Propter gemmarum naturam primitivam est subgen. *Protiteae* KIMURA subjungenda.

Hab. in Sina centrali.- - Specimen visum : " Prov. Hunan : prope urbem Tschangscha, secus viam fer-ratam trans Lingkwantu Bö-gaho usque; alt. s. m. ca. 30 m.", (HANDEL-MAZZETTI n. 11597 ♂ 17 Mart. 1919, in Herb. Hort. Bot. Novebora-censis).

48) **Salix madagascariensis**[1] BOJER ex ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 15, t. 1, fig. 12 (1867) (Monogr. Salic.) ; in DE CANDOLLE, Prodr. XVI. 2, p. 198 (1868). - PAX in ENGLER & PRANTL, Natürl. Pflanzenfam. III. 1, p. 36 (1894). GÄRTNER, Vergl. Blattanat. zur Systematik der Gattung Salix p. 20 (1907). BAUER, Die Blattanat. der pleiandrischen Weiden p. 23,40 (1909). - DAVY in Jour. Eco-logy X. p. 71 (1922).

Syn. *Salix australis* HILS & BOJER ex FRIES, Nov. Fl. Suec. Mantissa I. p. 77 (1832).

TRAUTVETTER, Salicetum in Mém. Prés. Acad. Sci. Pé-tersb. Savants Étrang. III. p. 622 (1836).

HERB. MUS. PARIS

Salix madagascariensis Bojer

Fig. 5. *Salix madagascariensis* BOJER ♀.

[1] Hic gratias quam maximas ago cl. Dr. R. METMAN, conservatori Musei Nationalis Historiae Naturalis Parisiensis, qui mihi summa liberalitate hanc raritatem commodavit.

Fig. 5, 6. Ad descriptionem ab ANDERSSON datam adde : — *Ramuli* annotini fusci cicatricosi minutissime cinereo-pubescentes. *Gemmae* ovatae obtusae brunneae, latere carinatae, dorso et latere puberulae, ventre gla-
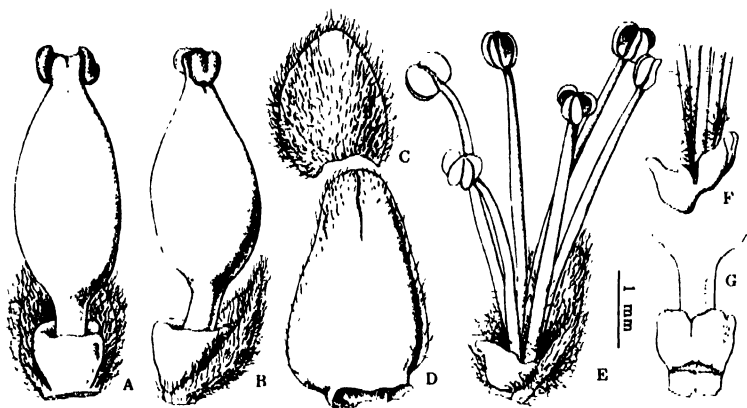
Fig. 6. *Salix madagascariensis* BOJER.   *A* Flos ♀.   *B* Idem a latere.
C Bracteola ♀ floris a dorso.   *D* Gemma sterilis a ventre.   *E* Flos ♂.
F Glandula cum basi androecii a latere.   *G* Glandula cum stipite ovarii a
dorso, resecta bracteola.

brescentes, circiter 3–4 mm longae 1.7 2.5 mm latae. Perula gemmalis latere ventrali supernis partibus exceptis connata, partibus liberis imbricatis 1/3 1/5 totae longitudinis. *Cataphylla* prima adaxialia, secunda abaxialia. *Folia* adulta primo dilute glauca demum pallidiora, supera facie stomatifera. *Amenta* ♀ oblongo-cylindrica, rhachidibus tomentosis, subrariflora, 1.3 2.5 cm longa, ramulos pubescentes folia ceteris similia gerentes 1.2– 3 0 cm longos terminantia. *Bracteolae* ovatae apice obtusissimae, intus tomentosae, extus superne glabrae ceterum tomentosae, flavidae, deciduae, in expansione circiter 1.8 mm longae 1.5 mm latae. *Glandula* carnosa cyathiformis stipitem ovarii perfecte cingens, margine truncata leviter undulata, ventre circiter 0.8 mm longa ac lata, dorso saepe emarginata et leviter fissa. *Ovaria* ovato-ellipsoidalia glaberrima sub anthesi 2.5–2.7 mm longa circiter 1.5 mm lata ; stipitibus glaberrimis vel basi parce puberulentibus, sub anthesi circiter 1.2 mm post eandem 2 mm longis ; stylis crassis brevissimis circiter 0.3 mm longis. *Stigmata* lateralia integra satis brevia. *Ovula* in quaque placenta 8–10. *Amentorum* ♂ tantum fragmenta visa : *Bracteolae* ut in ♀. *Glandula* cyathiformis basin androecii omnino cingens, margine undulatim plurilobulata. *Stamina* 6 7, filamentis

inaequilongis 2.5–4 mm longis, basi pilosis et leviter connatis; antherae luteae orbiculares circiter 0.4 mm longae ac latae.

Hab. in Madagascaria.— Specimina visa: " ♀ n. 1659. Vignier et Humbert. L'Itasy. District de Kitsamby. 27. 11. 1912 " & " ♂ Environs de Betafa. 7131. Perrier de la Bathie."

49) *Salix* subgen. *Protitea* sect. **Madagascarienses** KIMURA sect. nov.

Syn. *Salix* tribus A. *Salices Pleiandrae* a. *Tropicae* stirps III. *Salices africanae v. S. octandrae* ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. 1, p. 10 (1867) (Monogr. Salic.) pro parte.

Salix A. *Salices Pleiandrae* 1° *Tropicae vel subtropicae* § 3. *Africanae v. octandrae* ANDERSSON in DE CANDOLLE, Prodr. XVI. 2, p. 196 (1868) pro parte.

Frutices vel arbores humiles (ex ANDERSSON). Gemmae ovatae, perulis ventrali latere superne *liberis et imbricatis*, inferne connatis. Folia lanceolata, supra stomatifera, exstipulata, petiolis eglandulosis. Amenta foliato-pedunculata, subrariflora. Bracteolae ovatae tomentosae concolores, in ♀ deciduae. Fl. ♂ : Glandula cyathiformis basin androecii cingens, margine undulatim plurilobulata; stamina 6–7, filamentis basi pilosis et leviter connatis; antherae luteae. Fl. ♀ : Glandula cyathiformis, stipitem ovarii cingens, margine undulato-truncata, dorso leviter fissa. Ovaria glaberrima distincte stipitata, stylis minimis crassis. Stigmata satis brevia integra crassa. Ovula in quaque placenta 8 10.— Typus: *Salix madagascariensis* BOJER.

A ceteris sectionibus subgeneris *Protiteae* differt perulis gemmarum ventrali latere non omnino liberis sed inferne connatis. Species quaedam subgeneris *Euiteae* habent rarissime perulas gemmarum summo apice fere liberas et sulcum angustum formantes. Margines autem hujus sulci semper incurvati, nequaquam imbricati ut in *Protitea.* Confer T. R. RESVOLL, Über die Winterknospen der norwegischen Gebirgsweiden, Nyt Mag. f. Naturvidensk. XLVII. 4, p. 333–334, Fig. 12 (1909).

50) *Salix* subgen. *Protitea* sect. **Glandulosae** KIMURA in Tokyo Bot. Mag. XLII. p. 68 (Jan. 1928).— NAKAI, Fl. Syl. Korea. XVIII. p. 76 (1930).

Syn. *Salix* sect[1]. *Pentandrae* (non DUMORTIER) SEEMEN in ENGLER, Bot. Jahrb. XXI. Beibl. LIII. p. 56 in nota ad *S. glandulosam* (1896); ibid. XXIX. p. 276 (1900).— SCHNEIDER in SARGENT, Pl. Wilson. III. p. 98 (1916) p. p.-- K. S. HAO in FEDDE, Rep. Sp. Nov. Reg. Veg. Beih.

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[1] SEEMEN ill. significat " Gruppe ".

XCIII. p. 46 (1936) (Synop. Chinese Salix).

Salix A. *Didymadeniae* a. *Pleonandrae* α. *Brachystylae* I. *Pentandrae*
SEEMEN, Salic. Jap. p. 15 (1903).

Salix sect. *Glandulosae* NAKAI in Bull. Soc. Dendr. France no. 66, p.
7 (1928).

Salix sect. *Wilsonianae* K. S. HAO in FEDDE, Rep. Sp. Nov. Reg.
Veg. Beih. XCIII. p. 41 (1936) (Synop. Chinese Salix). **Syn. nov.**

51) **Chosenia** NAKAI in Tokyo Bot. Mag. XXXIV. p. 67 (1920); in Jour.
Arnold Arb. V. p. 73 (1924); in Bull. Soc. Dendr. France no. 66, p. 5
(1928); Fl. Syl. Korea. XVIII. p. 58 (1930).— KOMAROV in JACZEWSKI,
Mél. Bot. Borodine Jubil. LXXX. p. 277 (1927).— KIMURA in Tokyo Bot.
Mag. XLII. p. 290 (1928); in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI.
4, p. 394 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.).—
KOMAROV & KLOBUKOVA-ALISOVA, Key Pl. Far East. Reg. USSR p. 418
(1931).—NAZAROV in KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 22, 23
(1936); in FEDTSCHENKO, Fl. Transbaical. p. 223, fig. 124 (1937).

Syn. *Salix* tribus A. *Salices Pleiandrae* b. *Temperatae* stirps VII. *Sali-
ces fragiles s. S. albae* ANDERSSON in Kongl. Svensk. Vetensk. Akad.
Handl. VI. 1, p. 41 (1867) (Monogr. Salic.) quoad *S. macrolepidem.*

Salix A. *Salices Pleiandrae* 2° *Temperatae* § 7. *Fragiles v. Albae* AN-
DERSSON in DE CANDOLLE, Prodr. XVI. 2, p. 209 (1868) quoad *S. macro-
lepidem.*

Salix sect. *Pentandrae* "DUMORTIER" SCHNEIDER in SARGENT, Pl.
Wilson. III. p. 98 (1916) p. p.

Salix sect. *Amerina* (PLIN.) "DUMORTIER" FLODERUS in Arkif Bot.
XX. A. no. 6, p. 57 (1926) p. p. **Syn. nov.**

Salix sect. *Chosenia* (NAKAI) FLODERUS in Arkif Bot. XXV. A. no.
10, p. 2 (1933). **Syn. nov.**

Salix sect. *Dodecandrae*[1] K. S. HAO in FEDDE, Rep. Sp. Nov. Reg.
Veg. Beih. XCIII. p. 45 (1936) (Synop. Chinese Salix) p. p. **Syn. nov.**

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[1] Errore *Dodocandrae* scriptum.
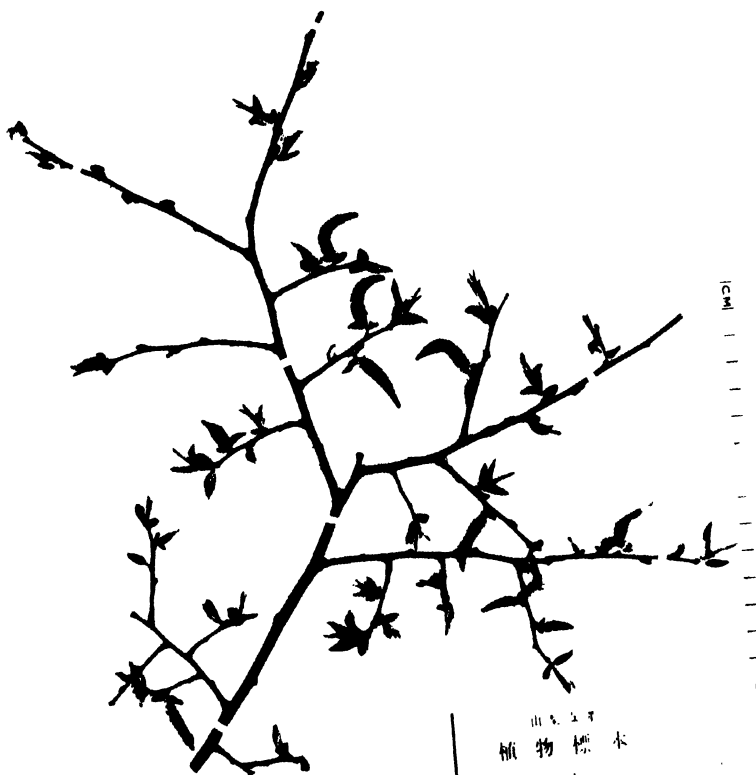
A. KIMURA: Symbolae Iteologicae V.

A. KIMURA: Symbolae Iteologicae V.

A. KIMURA: Symbolae Iteologicae V.

A. Kimura: Symbolae Iteologicae V.

EXPLICATIO TABULARUM

TAB. VI.

Salix Ikenoana KIMURA, Typus.
Ramuli amentiferi.

TAB. VII.

Salix Ikenoana KIMURA, Typus.
Ramuli cum foliis adultis.

TAB. VIII.

Salix rupifraga KOIDZUMI, Typus ⚥.
Ramulus amentifer.

TAB. IX.

Salix rupifraga KOIDZUMI, Typus ⚥.
Ramuli cum foliis adultis.

EFFECT OF SOME SALT SOLUTIONS ON THE PARTHENO-GENETIC MEMBRANE FORMATION OF SEA URCHIN EGGS

BY

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(Received June 6, 1938)

It has been shown by LOEB ('15) that the spermatozoa of a sea urchin will not fertilize eggs in a neutral m/2 NaCl solution or in any combination of NaCl, $MgCl_2$ and KCl, or even in the concentrations in which these salts are found in sea water; but that if $CaCl_2$ is added to such solutions, or to combinations of them, fertilization will take place. The addition of a small quantity of NaOH to salt solutions increases the fertilizing power of the sperm. These facts show that the chemical composition of the medium in which the egg and spermatozoa are placed have a direct influence on the fertilization reaction. It is also a well known fact that with the method of artificial parthenogenesis it is possible to induce the formation of the fertilization membrane. The author was interested in the particular point as to whether or not the parthenogenetic membrane formation would be affected by the chemical composition of the medium in the same way as in normal fertilization? The present paper deals with the results of his experiments on the membrane formation of sea urchin eggs in some salt solutions by previously activating them with an isotonic urea solution.

MATERIAL AND METHOD

A sea urchin, *Strongylocentrotus pulcherrimus* (A. AGASSIZ) was collected at Matusima Bay, and stored at 9°C in an ice box of the laboratory in Sendai. Eggs were cut out of the ovary every time before an experiment was made. For the parthenogenetic activation the author employed 1 mol solution of urea, which is approximately isotonic to sea water (MOTOMURA '34). He believes that the urea solution is better suited to the purpose than the usual method with butyric acid sea water, because the former is efficient and is the same pH as sea water. HERBST's artificial

sea water, Ca-free sea water, Mg-free sea water, isotonic solutions of
NaCl, KCl, MgCl₂, CaCl₂ and of urea were regulated to pH 8.3 by using
phenol red as an indicator. Experiments were carried out at room tem-
perature of 15° to 18°C.

RESULTS

Unfertilized eggs were first washed with 1 mol solution of urea for 3
to 10 seconds and then transferred into the solutions to be tested. After
three minutes eggs with the parthenogenetic fertilization membrane were
counted and the aspect of the membrane was noted. As a result, un-
fertilized eggs of the sea urchin formed the fertilization membrane parthe-
nogenetically in the simple salt solutions or in their mixture, when they
were previously washed with the urea solution for an adequate time. The
optimum time of washing and the percentages of eggs forming the mem-
brane are given in the following table.

TABLE 1

Parthenogenetic membrane formation in salt solutions

Solutions	Optimum time of treatment with urea	Percentage of eggs with the membrane	Toughness of the membrane	Hyaline membrane	Nuclear change
HERBST's sea water [1]	5 seconds	100%	+++	present	+
Ca-free sea water [2]	4 ,,	92 ,,	+	absent	+
Mg-free sea water [3]	6 ,,	86 ,,	+	,,	+
m/2 NaCl	5 ,,	100 ,,	+	cytolys.	cytolys.
m/2 KCl	4 ,,	85 ,,	+	absent	+
m/3 CaCl₂	5 ,,	92 ,,	++	,,	+
m/3 MgCl₂	4 ,,	83 ,,	++	,,	+

1) H_2O ....1000.0 cc.   2) H_2O ....1000.0 cc.   3) H_2O ....908.0 cc.
   NaCl ....26.3 gr.   NaCl..........26.9 gr.   NaCl .. ..26.3 gr.
   KCl ....0.7   KCl............0.7   KCl 0.7
   $MgSO_4(7H_2O)$..11.9 $MgSO_4(7H_2O)$ ..11.9 $CaCl_2(2H_2O)$ ..1.5
   $CaCl_2(2H_2O)$ ....1.5 $NaHCO_3$ 0.45
   $NaHCO_3$........0.45

In the HERBST's artificial sea water the membrane was easily formed.
By washing with the urea solution for 3 seconds the membrane was for-
med in 64% of the eggs, for 4 seconds in 76%, for 5 seconds in 100%,
for 6 seconds in 91% and for 7 seconds in 63%, respectively. In mono-
valent cations, especially in KCl, a high percentage of membrane forma-

tion was obtained only within a narrow range of time of urea treatment. But as is shown in the table, it is a noticeable fact that the optimum time of the urea treatment was nearly the same for all solutions used, ranging between 4 and 6 seconds.

The normal thickening of the parthenogenetic membrane was observed in the HERBST's artificial sea water, while, on the contrary, the membrane remained tender in other solutions. It was slightly thick in divalent cations in comparison with monovalent cations. In some cases, especially in the latter the membrane sank to the surface of the egg soon after its elevation. The nuclear membrane of the egg disappeared in the solutions of KCl, $MgCl_2$, $CaCl_2$, HERBST's, Ca-free and Mg-free sea water an hour after the immersion. And after 12 hours the egg showed a very irregular outline, which is suggestive of the egg activated with the butyric acid sea water without after treatment with hypertonic sea water. In NaCl the egg cytolysed within an hour. The hyaline membrane was never formed in the above-mentioned solutions except in the HERBST's artificial sea water.

DISCUSSION

The experiments mentioned obove showed that when the eggs of a sea urchin are previously activated with the urea solution, they form the fertilization membrane parthenogenetically even in the simple salt solutions of monovalent and divalent cations. The mechanism of this phenomenon seems to be different from the cases cited in the introduction, and it is rather comparable with the results obtained by HOBSON ('32) and by SUGIYAMA ('38). HOBSON showed that if eggs are fertilized in normal sea water and transferred within one minute to Ca-free sea water the fertilization membrane rises as usual. SUGIYAMA comfirmed HOBSON's results with Ca-Mg-free sea water instead of Ca-free sea water. Taking these facts into consideration the author is inclined to believe that if eggs are stimulated sufficiently either by spermatozoa or by the urea solution, they form the membrane in the sea water salt solutions.

The mechanism of the hardening of fertilization membrane is not known up to the present time, but some hypotheses about it were suggested. HARVEY ('10) was of the opinion, that the hardening of the membrane is presumably an oxidation and comparable to hardening of silk in the air. HOBSON ('32) found that the changes in the properties of the membrane after it has left the surface of the egg are dependent

on the presence of Ca in the sea water. SUGIYAMA ('38) also showed
that the membrane is hardened soon after its elevation not only by Ca
but also by some other divalent cations, such as Sr, Ba and Mg. In the
experiment here described a complete hardening of the membrane was
not observed in CaCl₂ and in MgCl₂. This fact shows that the process
of membrane formation in simple salt solutions is more complicated than
that in the solutions in which the salts are more nearly balanced, except
divalent cations. In the authors estimation, it is probable that the activi-
ty producing the substance — membranogen, with which the thickening
of the membrane proceeds, will be affected by the former solutions. If
the substance is not secreted enough, thickness of the membrane will not
reach a normal condition.

SUMMARY

1.   Formation of the parthenogenetic fertilization membrane of sea
urchin eggs in isotonic salt solutions and in their mixtures was reported.
2.   The thickening of the fertilization membrane was incomplete in all
solutions except in the HERBST's artificial sea water.
3.   The hyaline membrane was not formed in the unbalanced solutions.

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        thenogenesis. Sci. Rep. Tohoku Imp. Univ. Biol. **9**, 33.
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        urchin eggs. Journ. Fac. Sci. Imp. Univ. Tokyo Zool. **4**, 501.

EARTHWORMS OF KOREA. I.

By

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(With 17 figures)

Contents.

Introduction.

During the last five years, thousands of worms including many which were presented by my friends, were collected from localities covering the whole peninsula of Korea, as well as from various parts of Japan and Manchoukuo. The materials are mainly of the genera *Pheretima* and *Drawida*, and some belonging to the family Lumbricidae, among of which the genus *Pheretima* comprises the largest part. Of these three groups, the genera *Pheretima* and *Drawida* collected during 1933–36 in Korea are treated with in this paper. About a dozen species which were previously reported during the course of these years, are omitted here.

All figures, except those with special notes, are camera lucida drawings.

Collecting season in Korea.

In Korea, the collecting season is almost limited to the period from the beginning of April to the end of November. But, as the peninsula extends from Manchoukuo, where the climate is continental, southwards towards Kyûshû which lies across the Korean Strait, where the climate is warm, there are noticeable differences in climate between the northern and the southern regions of the peninsula. Also, owing to the difference of oceanic currents, a slight difference is found between the two sides of the peninsula, the west side being affected by a warm current and the east side a cold current. In the south-western corner and its adjacent islands, including Quelpart Island, worms can be collected almost throughout the year, and even in the coldest season, of Janauary and February, collection is not as difficult as in the other regions. In the coldest season

of the warmest region the worms penetrate into the ground only about 20 cm, while in the other regions they are found at a depth of more than 60 cm, and in the north central part of the peninsula the usual depth is more than 100 cm. Mr CHI KYÔ SHOKU dug to examine the depth where the worms live, on January 11th, 1936 (temperature −12.3°C), in Keijô; and found worms living at the depth of about 110 cm, these comprised 23 clitellate and 40 aclitellate and juvenile individuals of *Eisenia foetida*.

Most of the earthworms are hibernant, so collection should be made throughout the year. Unfortunately, most of the materials presented in this paper belong to the August-specimens. But, there are found annual worms such as *Ph. hilgendorfi*, *Ph. agrestis*, *Ph. vittata*, and some others which reach maturity after August. The growth rate of the first mentioned species was previously reported upon ('37).

It may be difficult to secure at arbitrary times, fully mature worms of the genus *Drawida*, but such specimens as of *Dr. gisti* were usually found throughout the collecting season, and in the period from June to August copulated worms of this species were often observed in their habitat.

Distribution.

For the general discussion of the distribution, further survey is necessary. From a single locality, such as Kôryô where there is an ancient sanctuary due to the presence of the mausoleum of the Ri dynasty, about twelve endemic forms were found, while from a number of localities scattered over the peninsula, most of which are either in town or village or harbour or suburbs, only about an equal number of endemic forms are recorded in the presented paper. Collections should be made in isolated localities as Kôryô, or in localities far apart from towns in order to know the true distributions of them.

I. Genus *Pheretima*.

As previosly stated ('36), *Ph. carnosa* is found even in the northern region of the peninsula except in its highlands. *Ph. hupeiensis* is also found in the northern region. But these two species are rarely found in the same spot, although, as peregrine forms, they make the northern limit in distribution of the genus *Pheretima*. These two species together with *Ph. hilgendorfi* and *Ph. agrestis* form the largest part of the materials obtained in Korea. *Ph. hilgendorfi* may possibly breed by self-fertilization as previously reported ('37) (similar phenomena of both *Ph. agrestis* and

Ph. vittata shall be reported at a later date). Special breeding habit as just mentioned is naturally much more convenient for their wide-distribution as peregrine forms, since by human-agency of a single worm or of a single cocoon (usually containing a single larva) it can then propagate its descendants in a new locality. *Ph. diffringens* (= *Ph. heterochaeta*), a form of world-wide distribution is not so abundant as the above mentioned species, and is evidently somewhat restricted to the south of central Korea.

Ph. aggera is widely distributed, though small in individual number in each locality, in the south of the line from Heijô to Eikô near Genzan. This line corresponds to the northern boundary of the endemic species in Korea, and at the same time, in the whole area of the genus. Although without an opportunity to examine any types of *Ph. tschiliensis*, if *Ph. aggera* is a synonym of the former species, it becomes a peregrine, and subsequently the northern boundary falls on the line from Shinkei (Kôkai-dô) to Mt. Kongô (*Ph. sp.*, an endemic species will be reported from there at a later date). Many endemic species are expected to be found in the south of this line.

II. Genus *Drawida*.

Twelve species of the genus have been recorded from north-eastern Asia, which is remote from the two principal regions of the genus, the southern and the north-eastern regions of India. Of these twelve species, *Drawida japonica* is, as is well-known, a peregrine; *Dr. anchingiana* was recorded from central eastern China and also from Quelpart Island; *Dr. gisti* from north China and also from central Korea (refer to part on systematics). All of the remaining nine and the new species of *Dr. koreana* and *Dr. keikiensis* are perhaps endemic to China, Korea, or Japan.

Dr. japonica is abundantly found in almost every part of the peninsula, including Quelpart Island, except in the far northern highlands and the primitive, non-cultivated land in the central and the southern regions. *Dr. nemora* is possibly a hilly earthworm, and has been found mainly in the northern part of the peninsula and appears to be common in the localities along the two rivers, the Ohryokkô and Tomankô; these two localities are out-side the *Pheretima*-region. *Dr. koreana* is also found mainly in the localities out-side the *Pheretima*-region. From the distribution of the genus *Drawida* in ancient times, it is noteworthy that *Dr. nemora* is rather commonly found out-side the *Pheretima*-region, and especially near the summit of Mount Kambô, at about 2,500 meters in altitude, where the ground is covered with snow in the long period of

September-June; and that according to the collector's saying (Mr RYÛHON SAITÔ) both places, Mt. Kambô and Akashima (a small island of a primitive land, situated at the north-eastern corner of the peninsula not far from Yûki) where this species was found, are very similar in ecological condition but greatly different in altitude. *Dr. nemora* is a relict form which may be distributed widely in east-northern Asia.

Pheretima-species without male pores.

In the genus *Pheretima*, characteristics of the male genital terminalia of XVIII are of major importance in specific characterization. As previously reported ('37) fifteen or sixteen (if *Ph. levis* and *Ph. irregularis* are specifically distinct from each other) species in the genus are destitute of male pores, and most of them are found in both Japan and Korea. In two certain species such as *Ph. soulensis* and *Ph. shinkeiensis*, even if the general characters simulate each other and the general appearance of the male pores is quite different from each other if present, when the pores are absent, difficulty is met with in specific distinction. *Ph. hilgendorfi* and *Ph. agrestis* both lack the male pores, but the former has two pairs of spermathecal pores with the midventral genital patch provided with numerous and minute genital papillae, and the latter species possess three pairs of spermathecal pores with the ventral and paired genital patches provided with no structures. Although, the other characters resemble each other more or less; of course, in those characters there are found many differences but of slight degree. Consequently, if the number of spermathecal pores are variable in both mentioned species as well as in *Ph. vittata*, the criteria for specific distinction become complicated. Fortunately, in the number of spermathecal pores of both species, at least in the present study, no variation was found, and specimens of both species lacking genital patch or patches were very rare. But, without statistical results on such organs, specific distinction by only minor differences is very difficult, and especially so when there is only a single specimen which lacks the genital patch or patches. A similar case was found in the identification of both *Ph. levis* and *Ph. irregularis*. Characters of both species must be studied on a sufficient number of specimens from various localities for reasonable determination.

'Spermathecal papillae' and lymph gland.

In species of the genera *Pheretima* and *Drawida*, the genital papillae

are found to be situated very close to the spermathecal pore, being inter-segmentally or either posterior or anterior to the pore. They are identical or not of the usual shape of the genital papillae which are situated segmentally being either presetally or postsetally. As the description of these two series of the genital papillae is complicated and liable to be confused in some cases, the former papillae are called in the present paper as ' spermathecal papillae ' and the latter as ' genital papillae '.

Position, shape and size of lymph gland are useful as a criterion in the genus *Pheretima*, and in the species presented in this paper they were found to be almost invariation. Even if slight variation was found, it was less marked than some other characters which are usually described, being never marked. They begin to appear: behind 15/16 or 16/17 or 17/18; behind the caecal segment; behind the middle portion of the body; and are often absent.

Acknowledgments.

Systematics.

GENUS DRAWIDA MICHAELSEN.

Drawida japonica (MICHAELSEN)

1931 *Drawida japonicus* f. *typicus*, MICHAELSEN, Peking Nat. Hist. Bull., II, p. 7.
1933 *Drawida japonica* (in part), CHEN, Contr. Biol. Lab. Sci. Soc. China, Vol. IX, Zool. Ser., No. 6, p. 189.
1935 *Drawida japonica*, GATES, Smithson. Misc. Coll., Vol. 93, No. 3, p. 3.

Drawida propatula GATES ('35) differs from the present species only in body size and presence or absence of the prolonged posterior appendices of the ovisacs. So, care examination was made mainly on these characters

of the materials at hand which are abundantly found almost everywhere the collections were made.   Body size is considerably variable, 32 × 3.2 mm–93 × 3.8 mm in the mature specimens; no larger ones were found among about a thousand specimens collected from about a hundred localitites. In general, it can be said that the specimens collected from villages rather remote from towns are smaller than those collected in the latter. Number of segments 126–158, and usually in the range of about 135–145. In shape of the body, the preclitellar
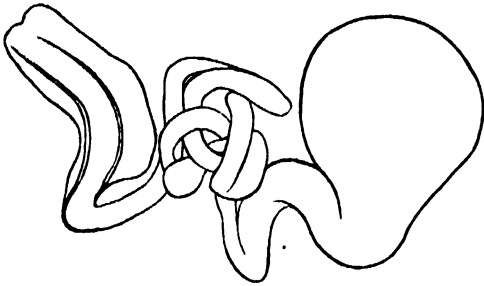
Fig. 1.   *Drawida japonica* (MICHAELSEN). Spermatheca, × ca. 48.7

region is rather ovoidal being slightly constricted in 9/10.

Male porophore is always represented as a small oval tumescence which is formed by elevated glandular skin of about posterior 1/5 of X, and is never so penis-like in shape as in *Dr. koreana*.

Spermathecal pores, in 7/8, between b and c, close to or just medial to c.

Genital papillae usually found on VII–XI, less frequently on XII and XIII also, sometimes totally absent.

Gizzards two in most cases, seldom three, in XII–XIII or –XIV.

Ovisac has always a prolonged posterior slender appendix; usually it extends posteriorly over XVII, and not rarely over XXV, and in extreme cases in the present examination into about LII; very seldom, only into XV or XVI. From this result, as Dr. GATES' sayings, it may be of important significance as a criterion for the present species. The prolonged posterior end may sometimes be overlooked when placed under the alimentary tract being free from the septa through its course.

A number of specimens belonging to the present species were also collected from Saishû, Quelpart Island, after my previous paper ('37) on the earthworm-fauna on this island.

Drawida gisti MICHAELSEN.

1931 *Drawida gisti*, MICHAELSEN, Peking Nat. Hist. Bull., V, Part 3, p. 8, figs. 2 and 3.

Keijô and its vicinity, a number of specimens, April–November, '34–'36.

Kinsen, Keishô-hoku-dô, two mature specimens, August, '35.

Description :

External characteristics :

Length 77–151 mm, generally about 100 mm, greatest diameter 4.5–6 mm ; number of segments 178-204, generally 180 190. Colour usually yellowish grey or sometimes fleshy, clitellum light russet, fleshy red or pinkish.

Setae paired, those on II usually very delicate. Anteriorly to about VII or VIII aa>bc or aa=bc, but in the region posterior to this, aa is considerably smaller than bc ; measuring in a specimen picked up at random, as follows : on III aa : bc=24 : 20, on VII 32 : 31, on X 25 : 34, on
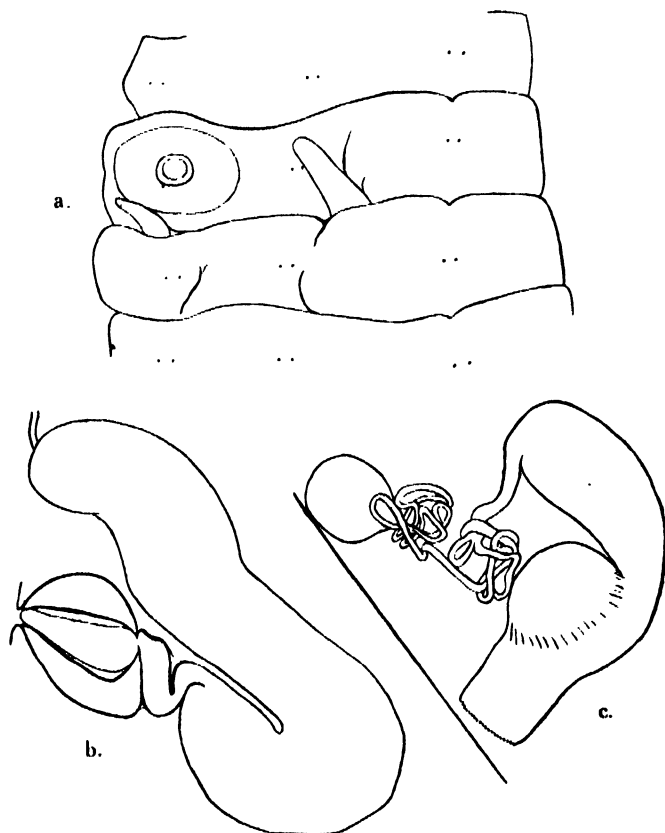
Fig. 2. *Drawida gisti* MICHAELSEN. a, ventrolateral view of male pore region, × ca. 12.6. b, terminalia of male genitalia, × ca. 12.6. c, spermatheca, × ca. 12.6.

XII 27 : 41, on middle portion of the body 27 : 40 ; dd a little larger than ½ of circumference.

Clitellum in X–XIV, thickly glandulated and swollen, easily distinguishable from rest of body by its glandulation and colouration. Glandulation of last segment sometimes slightly less distinct than the rest, and that of first segment may extend into a part of IX.

Nephridiopores in line with c ; frequently some of.them (in most cases those on clitellum) displacing dorsolaterally or dorsally or seldom ventrally.

Secondary male pores represented as large, wide slits in 10/11, between b and c, nearer to b. Heads of penes externally seen within slits, even when wholly withdrawn into the penial pouch. Penis blade-shaped, not sharply pointed, sometimes extended out directing anteriorly in various degrees, about 3.5 mm long and 0.6 mm thick at base when fully extended. (Fig. 2, a).

Female pores, one pair, slit-like, scarcely visible at anteriormost border of XII, in line with b.

Spermathecal pores, one pair, in 7/8, in line with c or just medial to c ; usually sunken into intersegmental furrow with a minute anterior lip.

Genital papillae usually found, but often absent ; circular, large, centrally with darkened spot, surrounding region gently elevated as a large oval area which is apparently glandulated and occupying greater part of anteroposterior length of segment. Occurrence in fifteen specimens picked up at random is as follows :

1. on VIII, left side only, just above ab ; on IX, left side only, lateral to b.
2. on both VII and VIII, midventral.
3. on IX, left side only, above ab.
4. on X, left side only, above ab.
5. on X, midventral.
6. on X, left side only, lateral to b.
7. on X, right side only, above ab.
8. on X, right side only, above ab.
9. on XII, right side only, above ab.
10. ..... 15. absent.

In general, they resemble in shape those of *Dr. japonica*, but are smaller in number and more frequently absent than in the latter ; found on VII–XII, but generally on VIII–X unpairly, usually in about ab-line or less frequently midventrally.

Internal anatomy :

Gizzards three in all of the examined twenty specimens, in XII–XIV, first one usually a little smaller than others and always distinct.

Testis sacs, one pair, large, ovoidal, suspended on 9/10, lying nearly equally in both IX and X or a little more in X, each slightly constricted at its lateral face by the septum 9/10, about 4 mm in anteroposterior length in fully mature specimens. Sperm-ducts long but fine, making a few masses of coils in front and behind 9/10; each finally entering directly into ental end of prostate.

Prostate large, consisting of three parts (Fig. 2, b); (1) sausage-like glandular portion, (2) shortly curved and thin but musculated duct, and (3) somewhat peach-shaped and thick-walled penial pouch. Glandular portion about 7–8 mm long and 1 mm wide, moderately warty on surface, usually curved at about its ectal 2/7 forming an *l*-shape; ectal end continuing to a thin but musculated duct which is about 1 mm long, usually once curved forming a *v*-shape when the penis is retracted into penial pouch, but straight when extended out; this duct continues to a slender long penis which fully occupies a musculated peach-shaped penial pouch. When the penis is extended out the duct tends to become straight through the inverted penial pouch. Penial pouch in normal condition about 1 mm wide and 1.5–2.2 mm long.

Ovaries large and tufted, fully occupying the dorsal part of XI. Ovisacs conical in shape, extending posteriorly into about XIII or XIV, each massive, fully containing yellowish ripen ova and rather smooth on surface, but in immature specimens almost empty and with much minute papilla-like swellings as stated by MICHAELSEN for his materials.

Spermathecae large, with its strium wholly situated behind 7/8 (Fig. 2, c). Ampulla small, spherical; duct rather sharply marked off from the former, fine and very long making a few masses of coils, finally entering with much increased thickness, directly into the broad ental end of a very large atrium. Atrium characteristic, sac-like, as large as several times the ampulla, about 4 mm long and 1 mm wide in broader ental portion; at about its mid-dorsal portion, always provided internally with a hard, musculated, elastic, somewhat yellowish, urn-shaped gland which is also a little larger than the diameter of the ampulla; this gland may be either markedly projecting dorsalwards into coelom or deeply sunken into the lumen of atrium indicating a small elevation there.

Thickly discoidal accessory glands are always found burried in the parietal wall corresponding to the external genital papillae.

Remarks:

The present species was first recorded by MICHAELSEN ('31) from Tsinan, Shantung, and Peiping, Hopei, China. Both localities are relatively not so distant from the present new ones.

Dr. cheni GATES ('35) was described by mistake by CHEN ('33) as the present species.

It is abunduntly found in Keijô and its vicinity, in farm-side, waste-land, and even in very poor soils such as in effloresced soil of granite.

Drawida nemora KOBAYASHI

1936 *Drawida nemora*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol. Ser., Vol. 11, No. 1, p. 141.

Keizanchin, Kankyô-nan-dô, three mature specimens, October, '33, by Y. MURATA.

Mt. Kambô, at the heights of about 2,000–2,500 m, Kankyô-hoku-dô, 10 mature and 5 immature specimens, September, '34, by R. SAITÔ and T. TEI.

Sempo, Kôgen-dô, two mature, four semi-mature, two juvenile specimens, and several fragments, August, '35.

Kôka, Keiki-dô, two semi-mature specimens, August, '35.

Myôkôsan, Heian-hoku-dô, a head of a juvenile specimen, July, '35.

Mt. Kambô, at the heights of about 2,300–2,400 m, two mature specimens, June, '36, by R. SAITÔ.

Yûki, Kankyô-hoku-dô, three mature specimens, August, '36.

Hekidô, Heian-hoku-dô, four mature and 16 semi-mature specimens, August, '36.

Kôkai, Heian-hoku-dô, one semi-mature specimen, October, '34, by J. MURAYAMA.

Kôshô, Heian-hoku-dô, one mature specimen, July, '35, by J. MURAYAMA.

(Akashima, kankyô-hoku-dô, several mature specimens, July, '37, by R. SAITÔ.)

Description:

Body size of the mature specimens is as follows: —

Locality	length mm	number of segments
Mt. Kambô	86–118	162–207
Kôka	114, 122	163, 172
Sempo	116	168
Yûki	144, 130	232, 217
Hekidô	142–185	198–222

Diameter up to 6.5 mm. In general, dorsal surface is deep blue on the preclitellar segments, and somewhat lighter postclitellarly; ventral surface light bluish. In fully mature specimens, the clitellum is not markedly swollen, and rather flat ventrally, dusty grey in colour; its midventral portion free from this coloration and rather non-glandulated, and almost similar to the rest of preclitellar segments in appearance. Clitellum in IX–XIII, sometimes its coloration and glandulation may extend posteriorly into a part of, or most of, XIV. On the postclitellar segments, epidermis, just lateral to b-lines and just medial to c-lines, is thinner than the rest, so they appear to be pale lines; ventral lines are always more distinct than the lateral. Setae on the clitellar segments are usually smaller than the rest. In immature specimens, setal bundles on preclitellar segments and sometimes also on upper portion of the postclitellar segments, are elevated tip-like, though setae themselves are not enlarged.

Spermathecal pores of the Kôryô-specimens (original) are in line with c or cd, while in most of the Yûki-, Kambô-, and Hekidô-specimens they are in line with d or frequently just lateral to d.

The secondary male pore of the mature specimens is a largely open slit situated on a moderate, sometimes slight, protuberance that involves the anterior margin of XI and the posterior margin of X; the interseg-mental furrow 10/11 is faintly (rather distinctly in immature specimens) indicated at the basal portion of the protuberance. The primary pore is always invisible externally.

In the original description I stated that the arrangement of the genital papillae which occur on the segments of VI–XIII seem to be regular. To detect whether it is regular or not, a re-examination was made on the specimens and also on the original specimens picked up at random. As is shown in the table, no definite rule holding for their arrangement was found; and besides, their actual positions are either presetal or postsetal or in the setal line though not indicated in the table. But, it may be said that they tend to occur, (1) on VII, VIII, and IX near the spermathecal lines, (2) on VI, X, XI, XII, and XIII near the male pore lines, and (3) to be a pair in each segment though not always strictly symmetrical in actual position. In younger specimens, the genital papillae are hardly recognizable, but the accessory glands have developed internally though very poorly. In several specimens also including some original ones, one or sometimes two spermathecal papillae were found just laterally to the spermathecal pore. Both spermathecal pore and papillae (or papilla) are placed in a crescentically formed slight depression

No.	VI		VII		VIII		IX		X		XI		XII		XIII	
	r	l	r	l	r	l	r	l	r	l	r	l	r	l	r	l
1	mc	mc	ld	ld	ld	ld	0	0	0	0	mc	mc	lb	mc	0	0
2	mc	mc	ld	ld	ld	ld	mc	mc	mc	mc	bc	bc	bc	bc	0	0
3	0	0	ld ld / bc		ld	ld	ld	0	mc	c	bc	bc	0	bc	0	0
4	mc	0	ld ld / mc	ld	ld ld / ld ld		ld ld / mc	ld	mc	0	bc	bc	bc	bc	mc	0
5	lb	lb	ld ld / lb lb / mc	ld	ld ld / mc	ld	mc	ld / d	mc	0	mc	mc	mc	0	0	0
6	0	0	mc	c	ld	ld	d	d	d	c	bc	bc	bc	bc	0	0
7	0	0	ld ld / mc mc	ld	ld ld / ld ld / d		d	d	c	c	bc	bc	0	0	0	0
8	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc	mc
9	ld	ld	ld	ld	ld	ld	ld	ld	mc	mc	mc	mc	0	0	0	0
10	0	0	mc	mc	ld	ld	d	d	d	0	0	0	0	0	0	0
11	0	0	0	0	0	ma	ma	ma	ma	ma	ma	ma	ma	ma	ma	ma
12	0	bc	ld	0	ld	ld	d	ld	mc	mc	mc	c	mc	0	0	0
13	0	0	ma	0	0	0	bc	0	0	ma	ma	ma	0	ma	ma	ma
14	mc	0	mc	mc	mc mc / bc	ld	c	c	d	0	0	0	0	0	0	0
15	0	0	mc	ld	0	0	d	0	0	0	0	0	0	0	0	0
16	0	0	0	0	ma	ma	ma	0	ma	ma	bc	bc	0	0	0	0
17	0	0	ld	ld	0	0	0	ld	0	0	0	0	0	0	0	0
18	mc	mc	ld ld / mc	ld	ld ld / ld		ld ld / mc ld	ld	mc	mc	mc	mc	0	0	0	0
19	0	0	0	ld	ld ld / ld ld		ld ld / ld ld		ld ld / ld ld		mc	mc	mc	mc	0	0
20	0	mc	0	mc	ma	ld	ma	0	0	0	ma	ma	b	0	0	0
21	0	0	mc	mc	ld	ld	c	c	0	0	mc	0	0	0	0	0
22	0	0	ma	ma	ma	ma	ma	ma	ma	0	ma	ma	0	0	0	0
23	0	0	0	0	0	ma	0	0	0	ma	ma	ma	ma	ma	0	0

1-7 Sempo-, 8 and 9 Kôka-, 10-22 Kôryô-specimens. ma indicates that a genital papilla is placed medially to seta a being either presetal or postsetal; mc medial to seta c; ld lateral to seta d; lb lateral to seta b; bc between setae b and c; c or d c- or d-line; r right, l left. VI, VII, VIII, etc. order of segment.

on the posterior margin of VII. Frequently even when such papillae are invisible externally, the accessory glands were found internally. Similar results were obtained in the examination of the genital papillae.

Nephridiopores are found at the anteriormost of each segment (except the head portion), and are minute, but may be easily recognizable, especially on the clitellar segments, indirectly from that the position is slightly depressed; on first two and a few segments of the end of body absent; usually on III–IX lateral to d, behind X in line with or iust lateral to d, but frequently they are displaced dorsalwards to be in a position either lateral or dorsolateral or rather close to middorsal line.

Gizzards are four or five in XIII–XVI or in XII–XVI; in most of the

former case the alimentary tract of XII is whitish and slightly musculated, but may not be said to be an actual gizzard.

In fully mature specimens, the internal genital organs, such as spermathecae, testis sacs, ovarian chambers, and ovisacs, are fairly large, fully occupying the genital segments. In such specimens, the testis sacs are frequently asymmetrically developed; that on one side is much larger and extends from a part of VIII or IX into XII or XIII, pushing the ovarian chamber and more the ovisac posteriorly (its posterior portion inserted into the ovarian chamber) and with slight septal constrictions through its course.

Prostates of all Kambô- and most of Hekidô-specimens are rather poorly developed and are burried in the parietes and covered by a sheet of the fine fibres derived from the lateral portion of the parietes, but do not differ from those of the other specimens in constituent structures and appear to be mature.

In every specimen, no kind of dilatation is found at the ectal end of spermathecal duct as in the original materials.

By brown masses of fine, long, complexly coiled tubes, the dorsal vessel behind gizzard is always thickly covered.

Remarks:

Each mentioned locality of this species is a hilly place relatively remote from large towns, as the original locality, Kôryô. It is of considerable interest that this endemic species of the genus *Drawida*, whose principal regions are both southern and north-eastern India, is found as a relic at the summit of Mount Kambô, at about 2,500 meters in altitude, and at the far east-northern region of the peninsula (N. L. 41° 43′).

According to a verbal communication of Mr R. SAITô, who made collections at both localities, Mt. Kambô and Akashima, the worms were found within wet roots of alpine plants such as *Pedicularis manshurica* MAXIMOWICZ in the former and under the decayed leaves on granite in the latter.

Drawida koreana, n. sp.

Kyôjô, Kankyô-hoku-dô, fifty-five semi-mature specimens (but, some may be rather immature), June '33.

Kyôjô, Kankyô-hoku-dô, several mature specimens, September, '33.

Kainei, Kankyô-hoku-dô, a single immature specimen, September, '33.

Ryûjin near Suigen, Keiki-dô, seventy-two mature and semi-mature, and

3 immature specimens, June, '34.

Tetsuzan, Heian-hoku-dô, two mature, 7 immature, and 2 juvenile specimens, August, '34.

Suigen, Keiki-dô, seven mature, 2 semi-mature, and one juvenile specimens, August, '34.

Tokugen near Genzan, Kankyô-nan-dô, one mature and one juvenile specimens. August, '35.

Kotei near Mt. Kongô, Kogen-dô, one mature and one juvenile specimens, August, '35.

Description:

External characteristics:

Colour, dark blue or dark reddish blue dorsally, lighter ventrally, clitellum reddish or pinkish in living condition.

Length 63–100 mm in apparently mature specimens, and usually 80–90 mm, greatest diameter in clitellar region 3–4 mm, number of segments 130–186, usually 140–170. Prostomium prolobous. Dorsal pores absent, and middorsal line not thinner than the rest. In shape of the body, I–IX rather cylindrical as in *Dr. nemora.*

Clitellum in X–XIII, not so distinctly swollen from the neighbouring segments as in *Dr. gisti*; but clearly distinguishable from the rest in glandulation and colouration. The ventral side of it is less glandulated, and the midventral portion is rather non-glandulated and tends to become somewhat flattened, especially on X and XI, and besides, the medial sides of the male porophores are slightly concave.

Setae short and closely paired. Those on II and on clitellar segments may be slightly smaller than the rest. Setal distance $ab=cd$; anteriorly to about VI aa larger than bc, on about VII–XV aa nearly equal to bc, behind this region, especially behind XX aa much smaller than bc; dd nearly equal to, or a very little larger than $\frac{1}{2}$ of the circumference. Just medial to c-lines and also just lateral to b-lines, the skin behind clitellar region is thinner, and each appears to be a distinct pale line.

Male pores, one pair, each on top of a moderately sized nipple-like or sometimes conical porophore, which is always formed by markedly elevated glandular skin near the posterior about $1/3$–$\frac{1}{2}$ of X, and is situated between b and c, much nearer to b. This porophore is always slightly projecting beyond 10/11 into anterior part of XI; anterior border of XI facing each porophore may be also slightly elevated (Fig. 3, a).

Female pores, one pair, minute, slit-like, scarcely visible in line with b, on quite anteriormost border of XII; rather easily visible in macerated

specimens.

Spermathecal pores, one pair, in 7/8, in c-line or just medial to c; each on a trifle tip which projects from the posterior edge of the preceding segment and is moderately sunken into the intersegmental furrow; mouth of this depression may be largely opened and its vicinity may be glandulated in mature specimens.

Genital papillae resemble in shape those of *Dr. japonica*, but much less in number, and not found infrequently; 1–4 in number if present, and irregularly placed on VII–XII, most frequently on VIII–X unpairly, and midventrally or in ab-line or between b and c.
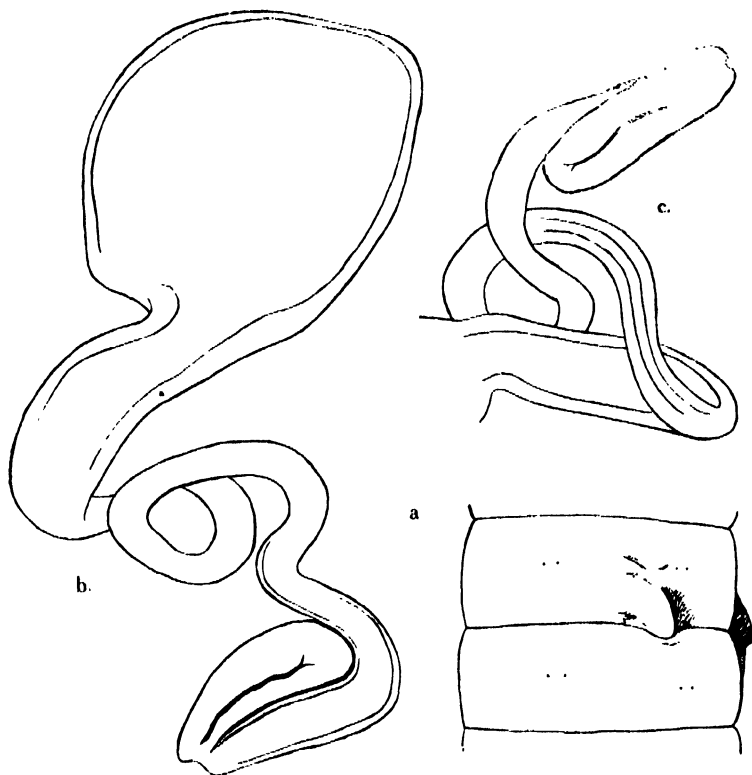
Fig. 3. *Drawida koreana*, n. sp. a, ventrolateral view of male pore region, × ca. 12.6. b & c, spermathecae, × ca. 48.7.

Internal anatomy:

Septa 5/6 and 8/9 moderately, 6/7 and 7/8 much thickened and somewhat musculated, the rest thin; all septa except 10/11 almost normally

inserted. Dorsal part of septum 10/11 displaced posteriorly to meet dorso-laterally with 11/12 in intersegmental space of 11/12.

Gizzards, two or three. each somewhat pot-shaped or barrel-shaped, small but strongly musculated and shining, in XII–XIII or –XIV. In every twenty of the Kyôjô and Ryûjin specimens their number was examin-ed : in the former mostly two (2 in fifteen cases and 3 in five cases) and in the latter mostly three (3 in fourteen cases and 2 in six cases). When three gizzards are present, the middle one is usually slightly larger than the others.

Testis sacs, one pair, moderate in size, each suspended by 9/10 the larger half lying in X. Its lateral face is usually constricted moderately by the septum, so it appears to be kidney-shaped. Those in mature specimens are massive, yellowish white, and granular on surface. Sperm-duct short, loosely twisted its larger part placed in IX, and piercing 9/10 into X, and entering into parietal wall just at anteromedial side of ectal end of the prostate. Prostate thick and short, about 2 mm long and 1 mm wide, usually compressed and weakly constricted at its ventral side in about middle portion, somewhat resembling testis sac in shape, but rather thumb-shaped in immature and also in semi-mature specimens, whitish to light brownish yellow and moderately warty on surface. Re-moval of its glandular investment reveals a relatively thick but short, tubular body.

Ovarian chamber in XI, formed by 10/11 and 11/12, both septa entirely fused dorsolaterally in intersegmental space of 11/12 (not reaching to middorsal), but separate by about one segment ventrally, also closed around oesophagus in an inverted U-shape. Ovaries fairly large, much tufted. Ovisacs situated dorsolaterally on alimentary tract, long and slen-der, moniliformed or distinctly constricted by each septum through its course, extending posteriorly into about XVIII, seldom behind this into XXII or XXIII and in front of this only into XIV or XV, and never extending so far back as in *Dr. japonica ;* yellowish in appearance fully filled with ripen ova ; ova about 5–10 μ in diameter. Posterior part of slender ovisac usually free from the septa, is situated in various places such as under or laterally to, gizzard or intestine, or pointing anterior-wards by reflection. Even when the ovisac is fully filled with ova and appears to be yellowish, the posterior end of it which may be only 1–2 mm does not contain ova and remains whitish in appearance compared to the rest.

Spermathecae, one pair, with its atrium wholly lying upon posterior

face of 7/8 (Fig. 3, b & c). Ampulla thin-walled, spherical (in immature specimens usually ovoid and empty) ; duct very short but very thick and slightly shining, not making a mass of coils in any parts, but loosely twisted, its ental portion much thicker than the rest, about two to three times of the latter. This much thicker and shorter portion resembles the ampullar duct and the following thinner and longer portion a diverticulum, in a spermatheca of the genus *Pheretima*. It is not sharply marked off from the ampulla ; ectally joining with ental and dorsal surface of the atrium, but its lumen not fusing with that of the atrium until near the ectal end of the latter. Atrium small and short, sac-like, always (at least in mature specimens) shorter than diameter of the ampulla, its ental end rounded but not enlarged ; when it is separated off from parietes, spermathecal opening may appear as a minute aperture at its ectal end.

Elastic, thickly discoidal accessory glands always found projecting through parietes into coelom, corresponding to the external genital papillae ; seldom, similar ones may be found internally even when papillae are not found externally.

Remarks :

The present species closely resembles *Dr. japonica* in many respects, but differs considerably in (1) general form of the body, (2) aspects of male porophore, and (3) length and thickness of spermathecal duct.

The region of I–IX is longer than that of *Dr. japonica ;* the shape of this region is cylindrical in the former and is rather shortly ovoid, being weakly constricted in front of clitellum in the latter ; anteroposterior length of each segment of this region is always, at least in equal-sized worms, longer than that of the latter.

Situation of the male pores resembles each other. But, the male porophore in this species is much larger and always somewhat nipple-like or rather penis-like in shape, while it is only a small oval tumescence in the latter. Proximal position of the porophore is also different ; in the former it is at about the posterior 1/3 or often almost 1/2 of X, while in the latter it is usually at about the posterior 1/5.

Length of spermathecal duct shows slight variation in both species. But, even in the comparison of the longest case of the present species with the shortest of *Dr. japonica*, the former is much shorter but much thicker than the latter and weakly musculated and shining, while in the latter it is rather fine through its entire length except for only both ental and ectal ends (Fig. 1, and Fig. 3, b and c).

The present species also resembles *Dr. propatula* GATES, but differs

mainly in body size, aspects of male porophores, and length and thickness of spermathecal duct. The differences between the present species and to either *Dr. propatula* or *Dr. japonica* appear to be more distinct than those between the latters.

Drawida keikiensis, n. sp.

Heiten near Suigen, Keiki-dô, two semi-mature specimens, August, '35.
Description :
External characteristics :

Length 40 and 54 mm, greatest diameter up to 2.5 mm, number of segments 154 and 138. Dorsal pores absent, but middorsal line appears slightly thinner than the rest. Prostomium prolobous. Colour in formalin, yellowish grey on both dorsal and ventral surfaces; clitellar coloration begins to appear, pinkish. Clitellar glandulation also begins to appear, in X–XIII; its glandularity extends ventrolateralwards, and is distinct around secondary male pores, but midventrad rather non-glandulated.

Setae short; those on II and clitellar segments not delicate, but may be a very little smaller than the rest. Behind clitellum they are slightly larger than the anteclitellar. Anteriorly to about V aa larger than bc, but behind this region aa nearly equal to bc; ab nearly equal to cd; dd nearly equal to, or slightly smaller than, 1/2 of the circumference.

Nephridiopores, minute, in line with c; some of them may be displaced dorsalwards.

Secondary male pores (Fig. 4, a), one pair, each represented as a relatively very large, transverse slit in intersegmental furrow of 10/11, formed by both posterior and anterior ends of X and XI. Medially it reaches to about a-line and laterally to near c-line. In one specimen from this slit a moderately sized, blade-shaped penis which is never pointed distally extends out anteromedially, as large as about 0.8 mm long and 0.3 mm wide at base when fully extended. Ventral epidermis of both X and XI around secondary male pores are thickened and gently elevated, the clitellar glandularity extending into there, but the midventral portion in this region is rather concave; setal zones of XII and XIII are also elevated though much faintly.

Female pores, one pair, invisible in these specimens externally, in line with b, in 11/12.

Spermathecal pores, one pair in 7/8, in line with c or just medial to c.

Genital papillae absent in both specimens.

Internal anatomy:

Septa 5/6 and 6/7 very much, 7/8 and 8/9 much thickened, the rest thin or delicate. Dorsal parts of 10/11 displaced posteriorly to meet dorsolaterally with 11/12 in intersegmental space of 11/12. The other septa almost normally inserted.

Gizzards, three in one specimen in XIII–XV, and four in the other in XII–XV; spherical in shape, strong, large relatively to the body size, and rather clearly constricted from one another to be moniform as a whole.

Last hearts in IX.

Testis sacs, one pair, small but rather massive, yellowish, each suspended by 9/10 a little larger part lying in X, faintly constricted at its lateral side by the septum. Sperm-duct very short and very fine, shorter than the length of prostate, wholly lying behind 9/10 and making there only a few twists within a sheet of connective tissue which is apparently derived from the ventral parietal wall, and stands in coelom to reach about to the proximal portion of the sperm-duct, and falling ventrally to the floor of the coelom in X where it penetrates into the parietes and passes laterally and then emerges into the coelom and at once may possibly enter into the ental end of the prostate. Unfortunately, in spite of my careful dissection the position where the sperm-duct enters into the prostate was not ·decidedly determined; but from their relative situation the above description was written. Prostate (Fig. 4, b), small, short but relatively broad, highly warty on surface, rather flattened dorsoventrally and a brownish tubular body visible within it under the microscope, ectally slightly curved anteriorwards and covering a part of dorsal surface of a large gland which is placed medially, entally not erected into coelom, but bent ventralwards and bound but not firmly, by a few muscle fibres to the ventral parietal wall in anterior part of X. Duct thin and very short, concealed by a small mass of tissue, so it appears that the prostate gland directly continues to the proximal end of the penis. Medially to the prostate is found a very large, firmly formed, glassy shining, ovoidal gland which is large as about 1/4–1/3 of the prostate, dorsally rounded and markedly projecting into the coelom but slightly concealed by the ectal part of the prostate, ventrally with the narrowed portion passing into the penis. Penis not so long, dorsoventrally flattened, hook-shaped, being anteromedially curved, distally never pointed. (Corresponding to the gland genital papilla was not found externally, but it may be found.)
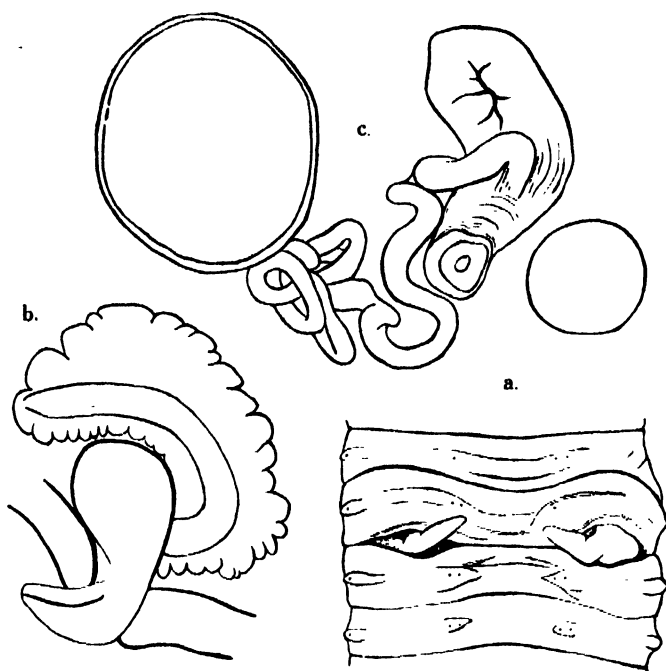
Fig. 4. *Drawida keikiensis*, n. sp, a, ventral view of male pore region, ×ca. 17.2. b, terminalia of male genitalia, ×ca. 23.3. c, spermatheca and accessory gland corresponding to spermathecal papilla, ×ca. 48.7.

Ovarian chamber in XI, formed by septa 10/11 and 11/12, ventrally separated by about one segment and dorsolaterally both septa fused in intersegmental space of 11/12, also around oesophagus forming an inverted U-shape. Ovisac long, slender, extending posteriorly into XVI in the shortest case or into XXII in the longest case, its anterior portion constricted by each septum, moniliform, empty and whitish in these specimens; thin wall of these sacs provided with much minute papilla-like swellings on its various parts. . . . perhaps they may disappear when sacs are filled with ripen ova as in the case of *Dr. gisti*. Ovaries large, much tufted.

Spermathecae, one pair, with its atrium wholly lying upon the posterior surface of 7/8. Ampulla large relatively to the body size, spherical; duct thin, moderate in length, not sharply marked off from the former, making a small mass of coils at its ental portion, ectalwards gradually increasing its thickness in a very small degree and loosely twisted there, finally entering into about ectal third of the atrium. Atrium somewhat

sac-like, nearly equal to, or a little longer than, diameter of the ampulla, placed laterally to ampulla being erected into coelom, its ental free portion rather rounded and slightly thicker than the rest. Just laterally to atrium is found a small, whitish, stick, and ball-like gland (Fig. 4, c).

Remarks :

The present species in some characters closely resembles *Dr. syringa* CHEN ('33) and also *Dr. cheni* GATES ; for instance, in the former species in the highly warty and small prostate, prolonged slender ovisacs, and absence of genital papillae, and in the latter species in some characters of the male organs. But, it differs from the former mainly in shape of penis and probably also in the structure of the other parts of the male organs, and in position where the sperm-duct enters into the atrium. The description of *Dr. syringa* is rather brief, so the differences and at the same time the resemblance between them cannot be clearly shown here. But, the differences above mentioned may be sufficient to specifically distinguish them from each other. From the latter species it is clearly distinguishable in many important characters.

GENUS PHERETIMA MICHAELSEN.

Pheretima irregularis (GOTO et HATAI)

1899 *Perichaeta irregularis,* GOTÔ and HATAI, Annot. Zool. Japon., Vol. III, p. 11.

Rensen near Keijô, Keiki-dô, a single clitellate specimen, posterior end appears to be regenerated, August, '35.

Description :

External characteristics :

Length 75 mm, greatest diameter 5 mm, number of segments 82. Colour in formalin, brownish dorsally, lighter ventrally, clitellum blackish brown. Prostomium, epilobous ca. 1/2, posterior demarcation distinct. First dorsal pore in 12/13 ; a non-functional pore-like marking found in 11/12. Clitellum entire, in XIV–XVI, without setae.

Setae beginning on II, moderate in size ; nearly equal in size throughout the entire length of the body ; both mid-dorsal and -ventral breaks rather distinct but slight. Setal number as follows : 24/III, 34/V, 36/VII, 39/VIII, 39/IX, 43/XVII, 41/XX, 43/XXX.

Female pore, single, midventrally on XIV.

Male pores, spermathecal pores, and genital papillae are all absent entirely.

Internal anatomy :

Generally, no septa especially thickened; 5/6 and 6/7 moderately thickened, 7/8, 10/11, and 11/12 thin, 8/9 and 9/10 absent.

Gizzard large; intestine beginning to swell in XV; caeca in XXVII, small but complicated, each with five (right side) or six (left side) finger-shaped secondary caeca, of which the dorsalmost is longest, extending anteriorly into about XXIII, the more ventral ones becomes gradually smaller.

Hearts in X quite vestigeal in size. Lymph glands small, found behind caecal segment caudalwards.

Seminal vesicles, two pairs in XI and XII, large but not meeting middorsally with one another, nearly equal in size and in shape, each with a small dorsal lobe; dorsal lobe of the anterior pair rather indistinct. Testis sacs, two pairs in X and XI, small; anterior pair ovoidal, widely separate from each other; posterior pair appears to be also separate but more closely placed than in the anterior pair; those in both segments are closely in contact with each other on each side. Pseudovesicles, one pair, moderate in size, behind 12/13.

Prostate glands and ducts are totally absent. Sperm-duct on each side terminating blindly with a bulbular swelling as in *Ph. agrestis*, in XVI on left side and in XVII on right side.

Ovaries in XIII, relatively large.

Spermathecae entirely absent.

Note on **Ph. levis** and **Ph. irregularis**.

Both *Ph. levis* and *Ph. irregularis* were described as new species by Gotô and Hatai about forty years ago from Japan, the former species for the specimens collected from Takahashi (Okayama Prefecture) and Kumamoto (Kumamoto Prefecture), and the latter from Uwajima (Ehime Pref.) and also Takahashi. So, it is presumable that a certain number of specimens belonging to both species were collected from the same locality, Takahashi, and possibly from the same spots, respectively.

Both species closely resemble each other in many important characters, or appear to be specifically almost identical with each other if the difference of presence or absence of the spermathecal pores (and also of spermathecae) were neglected which may show considerable variation intraspecifically as in *Ph. vittata*. But, we must remember that there are *Ph. hilgendorfi* or *Ph. agrestis*, in each of which in spite of lacking the male pores it has

	Body size	No of segment	No of setae	First dorsal pore	Male pore	Spermathecal pore	Genital papillae
irregularis by GOTÔ and HATAI	125×6	95	47/VII & VIII 61/XVII	12/13	0	0	0
Rensen-specimen	75+×5	82+	36/VII, 39/VIII 43/XVII, 43/XXX	12/13	0	0	0
Gifu-specimen	97+×5.5	87+	46/VII, 52/VIII 47/XVII, 46/XXX	12/13	0	0	0
levis by GOTÔ and HATAI	85×4.5	88	45/VII & VIII 48/postcl.	12/13	0	in 6/7 & 7/8	0 but with spth. ppll.
Hôsen-specimen	80×5	96	40-41/VII & VIII, 46/XXX	12/13	0	in 6/7 & 7/8	1 pair & spth. ppll.

Caeca	Seminal vesicles	Pseudo-vesicles	Testis sacs	Sperm-ducts end in	Sperma-theca	Lymph gland	Hearts in X
5 pairs XXVI	large	large	?	XIV-XXII	0	?	?
5-6 pairs XXVII	large	moderate	separate	XVI or XVII	0	small, behind XXVII	vestigeal
5-6 pairs XXVII	small	large	separate	XXIV or XIX	0	vestigeal	not found
5 pairs XXVII	?	very large	?	usually ant. XVIII	pyriform	?	?
5 pairs XXVII	moderate	small	separate	XIV or XVI	peach-shaped	small, behind XXVII	not found

always spermathecal pores. And also, some minor differences are found between the two species concerned.

Unfortunately, I have too few specimens, at present, of each species to discuss their validity here. I have a single specimen collected from Gifu (Gifu Pref.), Japan, which appears to be also identical to *Ph. irregularis*. Comparing these three specimens with the original descriptions of both species, as the above table.

(It is much regreted that no specimens belonging to *Ph. irregularis* were included among the materials collected by the good-will of Mr. M. TAKEUCHI from its original locality, Takahashi.)

Pheretima vittata (GOTO et HATAI)

1936 *Pheretima (Ph.) vittata*, KOBAYASHI, Jour. Chôsen Nat. Hist. Soc., No. 21, pp. 52-57.

Kinkai slightly apart from Fusan, Keishô-nan-dô, 50 clitellate specimens, August, '36.

Keijô, a number of juvenile, aclitellate, and clitellate specimens, June–October, '36.

Taiden*, Chûsei-nan-dô, a clitellate specimen, August, '36.

Tôrai*, Keishô-nan-dô, seven clitellate specimens, August, '36.

Fusan*, Keishô-nan-dô, an aclitellate specimen, August, '36.

The Korean specimens are much smaller than the Japanese; length 105–130 mm. Number of segments 88–99. Coloration less distinct in characteristic banded appearance than the Japanese materials. Male pores usually absent; seven out of fifteen Kinkai-specimens have those but unpairly in all of these, six cases on right side and one case on left side. Number of spermathecal pores are very variable (Keijô-specimens were used for the other experiments). Variation of occurrence of spermathecal pores is shown below.

```
1. ....2 pairs in 6/7 & 7/8 .................................30 specimens
2. ....1 pair in 6/7........................................ 3
3. ....a. 2 pairs in 6/7 & 7/8; left only in 5/6 ............. 3
       b. 2 pairs in 6/7 & 7/8; right only in 5/6............. 2
4. ....a. 1 pair in 6/7; left only in 7/8 ................... 2
       b. 1 pair in 6/7; right only in 7/8 .................. 2
5. ....1 pair in 7/8; right only in 6/7 ..................... 2
6. ....right only in 6/7 & left only in 7/8 ................. 1
7. ....right only in 6/7 & 7/8.............................. 1
8. ....left only in 6/7...................................... 3
9. ....totally absent ...................................... 1
                                                          ─────────────
                                                          50 specimens
```

Except for a few cases, one very small or two genital papillae are found close to each spermathecal pore. Their frequency of occurrence and their accurate situation are too variable to be described here.

All fifty specimens were opened.

When the prostates are absent, the sperm-ducts always blindly terminate with a bulbular swelling; terminals on both sides which are not always symmetrical are shown below.

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* These specimens are preserved in the Biological Institute of Tôhoku Imperial University, Sendai, Japan.

Side	Order of segment																				
R	XIV	XV	XIV	XV	XVI	XV	XVI	XVII	XVI	XVII	XVIII	XVII	XVIII	XIX	XVIII	pr.	pr.	XVI	XVII	XVI	XVII
L	XIV	XIV	XV	XV	XV	XVI	XVI	XVI	XVII	XVII	XVII	XVII	XVIII	XVII	XIX	XV	XVII	pr.	XIX	XVIII	XV
	1	2	0	1	5	4	10	4	6	3	0	2	0	2	0	2	4	1	1	1	1
	Number of specimens																				

As is clear from the above list they usually terminate anteriorly to XVIII.

Intestine begins to swell in XV; caeca in XXVII, each consisting of several finger-shaped secondary caeca. Hearts of IX are asymmetrical, one is regular in shape and the other is rudimentary, but both connect with the ventral vessel respectively; the larger is found on the right side in 29 cases and on the left side in 20 cases, and in one case on both sides as nearly equally small calibre and regular shape. Hearts of X not found in all specimens. Lymph glands small, seldom very small, found behind caecal segment caudalwards.

Seminal vesicles small, two pairs in XI and XII. Frequently quite small or rather vestigeal in size or somewhat resemble the pseudovesicles. Showing their approximate frequency as follows:

	usual size	smaller	vestigeal
anterior pair	21	15	14
posterior pair	23	12	15

That about one third of the specimens examined have the vestigeal seminal vesicles is noteworthy. Most of both usual- and smaller-sized vesicles are heavily infested by parasites, including a number of Gregarin cysts, while the vestigeal ones all are free or nearly free.

Spermathecae are usually found internally when the pores are visible externally. But, in sixteen specimens vestigeal ones were found. In two cases out of these sixteen, in spite of the fact that the pores were clearly recognizable externally, no trace of the spermathecae was found internally.

In all of the specimens examined, Gregarin cysts were found in various degree on the parietes and gut, around spermathecae, and within seminal vesicles, especially much in the last. But, only two specimens among the sixteen above mentioned were heavily infested.

Remarks:

As Dr. GATES informed me, some interesting facts will be obtained

in the study of the parasitic infestion in the juvenile specimens of this species.

The Korean specimens (also including those used in my previous work ('36)) are clearly different in some characters from the Japanese ones. The banded colouration is less distinct than the latter in which it is very clear and quite unique. The colouration of the former rather resembles that of *Ph. diffringens* though it is a very little more distinct than the latter. Is it only a local variation? According to HATAI ('29), in both materials collected from the northern and southern parts of Japan this banded colouration is equally distinct. As is shown in my previous paper and also in the present one, no case of having the spermathecal pores in 5/6 is found in the Korean specimens, while in the Japanese it is not rare in 5/6.

Pheretima koreana, n. sp.

Kunsan, Zenra-hoku-dô, seven clitellate specimens, August, '34.

Zenshû, Zenra-hoku-dô, four clitellate specimens, August, '34.

Description :

External characteristics :

Length 93-103 mm, greatest diameter 4.5 mm, number of segments 93-102. Colour (according to the note made when the worms were collected), dorsally brownish and ventrally lighter than the dorsal, clitellum light chocolate. Prostomium, epilobous ca. 2/3, somewhat V-shape but without posterior demarcation. First dorsal pore in 12/13, sometimes a non-functional pore-like marking may be found in 11/12.

Setae beginning on II, moderately large; ventral ones on about VI–XII or –XIII may be slightly enlarged and planted on faint ridges. In general, ventral setae are slightly larger than the dorsal, but in interval nearly equal to each other. Setal breaks slight but found throughout the entire length of the body, aa=1.4-2.2 ab, and zz=1.3-2.4 zy. Setal number as follows: 26–31/III, 37–41/V, 43-46/VI, 44–56/X, 43–52/XX, 18-24 between male pores.

Clitellum entire, in XIV–XVI, without setae.

Spermathecal pores rarely present, and male pores frequently absent. Occurrence of them is shown below :

Locality	No.	male pores		spermathecal pores	
		R	L	R	L
Kunsan	1	absent	absent	6/7	absent
	2	absent	absent	absent	absent
	3	present	present	6/7	5/6
	4	present	absent	absent	absent
	5	present	present	absent	absent
	6	present	present	absent	absent
	7	present	present	6/7	absent
Zenshū	8	absent	absent	absent	6/7
	9	absent	present	absent	absent
	10	present	absent	absent	absent
	11	present	absent	absent	absent

Male pores (Fig. 5, a) : Ventral surface of about XVII–XX is some-what flattened ; lateral sides of XVIII are slightly stretched lateralwards to form a triangle on each side where the epidermis is slightly thickened and light-coloured and the intersegmental furrows of 17/18 and 18/19 are rather indistinct. A pair of small oval shallow depressions which corre-spond to the secondary male pores are found on the setal line of XVIII and near the corner of the stretched triangle and being about 2/5 of the circumference ventrally apart. A minute tip bearing the primary male pore together with one or two indistinctly demarcated very small papillae found externally at the bottom of this depression when its mouth is opened. When the male pores are present, the genital papil-lae are always found (at least in these specimens). On medial margin of the depression, two or seldom one small but distinctly demarcated circular and centrally depressed genital papillae are placed, in a transverse row if two are present, presetally or· rather tangential to the setal line. When male pores are absent, ventral surfaces of XVII–XX are
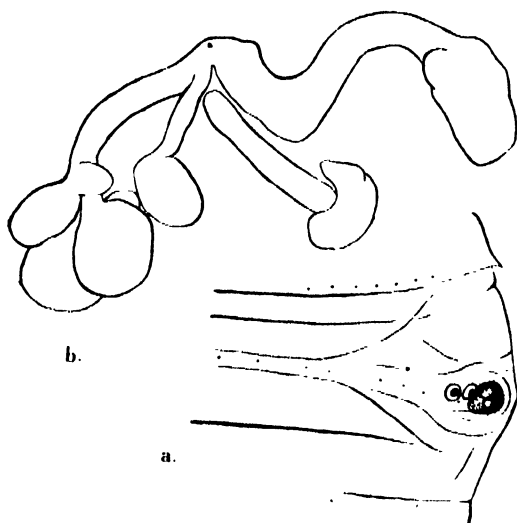
Fig. 5. *Pheretima koreana*, n. sp. a, ventral view of male pore region, ×ca. 12.6. b, an abnormal sper-matheca with an accessory gland, ×ca. 28.

not stretched and not flattened as in the former, and are equally rounded as in the rest of the body.

Spermathecal pores rarely present. The pore aspect is quite similar in all cases; it is minute, with simple lips on its medial borders, and laterally accompanied with a very small spherical spermathecal papilla.

Female pore, single, midventrally on XIV.

There are no more genital markings.

Internal anatomy:

In general, no septa especially thickened; 5/6 and 6/7 slightly thickened, 7/8, 10/11–12/13 thin, 8/9 and 9/10 absent.

Gizzard small, barrel-shaped. Intestine begins to swell in XV. Intestinal caeca originating in XXVII, small but complicated, each consisting of four to six finger-shaped secondary caeca, of which the dorsalmost is largest, extending anteriorly into about XXIV, the more ventral ones become gradually smaller. In one specimen, the caecum of the left side consists of six secondary caeca, but on right side no trace of the caecum was found.

Last hearts in XIII; hearts in X absent in all cases. Lymph glands small, found behind caecal segment caudalwards.

Testis sacs moderate in size; those in X forming ventrally a low V-shaped sac and those in XI a transverse sac; those of both segments in contact with each other. Seminal vesicles, two pairs in XI and XII, rather small, each oval, flattened, weakly vesicular on surface, with a small but distinctly marked off, smooth-surfaced dorsal lobe. Both pairs are nearly equal in size, but dorsal lobes of the posterior pair are usually larger than the anterior. Pseudovesicles, one pair, large, on posterior face of 12/13. Sperm-ducts on each side meeting at the posterior part of XII.

Prostates were always found when the male pores are visible externally. Glandular portion small or moderate in size, only confined to XVIII or XVII–XIX or XVIII–XIX, usually brownish yellow in colour and deeply incised into two portions in the region where the duct is connected with the former. Duct short and thin, looped in a U-shape with rather fine ental half and the thicker and shining ectal. The ectal end of the duct passes with slightly decreasing in thickness into the body wall to open directly to the exterior within the depression as a primary male pore. Prostate glands and ducts are totally absent when the male pores are not found externally. In such cases, the sperm-duct blindly terminates with a bulbular swelling as in *Ph. agrestis,* in some of XVI–

XVIII.   Quite close to the ectal end of the prostatic duct are found one
or two very small whitish accessory glands with fine but relatively long
stalk which correspond to the very small papillae within the male pore
depression.   Slightly anteriorly to these glands are found two, or seldom
one, larger brownish yellow (this colouration closely resembles that of the
prostate gland) accessory glands with moderately long and rather thick
stalk which correspond to the external genital papillae placed externally
out-side the depression.

Spermathecae are entirely absent when the pores are invisible ex-
ternally.   In every specimen having the pore externally, spermatheca was
found internally in the corresponding position.   But, those are variable
being quite different from one another in shape and also in size, and
appear to be rudimentary or abnormal.   Following descriptions were made
from three Kunsan-specimens.   In the first specimen : it is moderate in
size, ampulla small, thinly ovoidal and simply folded, containing a very
little whitish mass ; duct slender but longer than the long axis of the
ampulla, ectalwards becoming gradually thinner ; diverticulum short and
fine, about one third or less than the main portion, not dilated at its
terminal end.   In the second specimen : it is clearly abnormal, and much
smaller than that of the first specimen (Fig. 5, b) ; at first glance there
is no marked difference in size between the main portion and the diverti-
culum, and discrimination between them was only possible from the
difference of their inner structure ; ampulla very small, ovoidal, containing
a little whitish mass ; duct moderate in relative thickness and much longer
than the ampulla, about three times of the latter ; diverticular duct branch-
ing into two portions at its proximal, and the larger one is provided with
five variously sized very small swellings, of which the largest is rather
larger than the ampulla, each swelling appearing to contain a very little
whitish mass.   In the third specimen : the spermatheca is nearly equal in
size to that of the second specimen ; ampulla small, transversely thinly
ovoidal ; duct thin but slightly longer than the ampulla and from the
latter distinctly marked off ; diverticulum very large relatively to the size
of the main portion, its stalked portion is as thick as or a very little
thinner than the ampullar duct and the ental half is moderately distended
forming an elongated somewhat thumb-shaped seminal chamber.

Very close to the spermatheca if present, is always found a very
small whitish accessory gland with fine but long stalk corresponding to
the spermathecal papilla.   Whenever the spermatheca was pulled out to
separate the parietes, the spermathecal papilla is removed together with

the former and there is then found a large hole intersegmentally.

Remarks:

Leaving out of consideration whether it is normal or not, as of athecal forms of the genus *Pheretima* we can enumerate at present about a dozen species. Of these, the present species is closely related to *Ph. irregularis* and *Ph. levis*. But, from these, it is distinguishable mainly in the structure of the anterior male organs, and in frequent occurrence of the posterior male organs, and also in the general form of the body.

Pheretima kobayashii, n. sp.

Reisui, Zenra-nan-dô, a single clitellate specimen (but, its clitellar glandularity not yet complete), August, '34.

Description:

External characteristics. Length 91 mm, greatest diameter 4 mm, number of segments 110. Original colouration not recognizable, too faded. Prostomium epilobous, ca. 2/3. First dorsal pore in 12/13, distinct and functional.

Setae beginning on II, delicate in size; in general, preclitellar setae smaller than the postclitellar, so it is difficult to count their number on the former region. Both mid-dorsal and -ventral breaks found, but slight. Ventral setae are a little larger and more closely set than the dorsal. Setae between male markings on XVIII are obliterate, and some setal pits of them are scarcely recognizable under the microscope; perhaps they are entirely invisible in mature specimens. Setal number as follows: 41/III, 62/VI, 64/VII, 62/IX, 61/XX, 36(V), 37(VI), 37(VII) between spermathecal pores; (male setae about ten if present).

Male pores situated ventrolaterally on the (supposed) setal line of XVIII, about 1/3 of the circumference apart; each opened as a rather large aperture on a small circular papilla which is placed on the lateral part of the large genital marking (Fig. 6, a).

Ventral surface of XVII–XIX flattened, where the region, from the setal line of XVII to that of XIX (=2 segments) is slightly depressed forming a large quadrate area which appears to be glandulated (setae on XVIII on this area are obliterate, as described above). The genital markings are somewhat kidney-shaped, placed ventrolaterally on XVIII, anteroposteriorly extending through the whole segment and a little way over both intersegmental furrows of 17/18 and 18/19. The marking is slightly depressed into the parietes, and distinct in outline except the
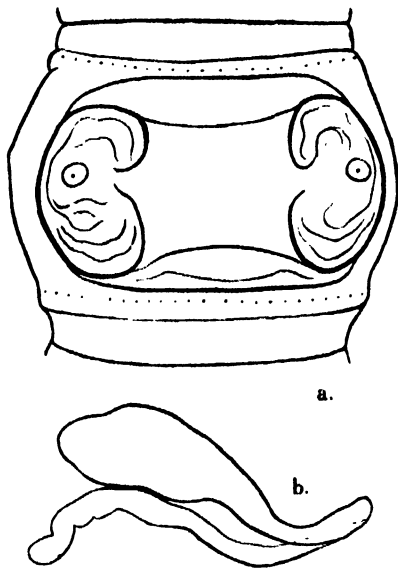
Fig. 6.　*Pheretima kobayashii*, n. sp.
a, ventral view of XVII XIX, ×ca. 12.6.
b, spermatheca, ×ca. 12.6.

medial middle portion. Its surface is not clearly even, but with a few indistinct irregular indentations; and on the lateral part of the surface is found a small circular protuberance centrally with a rather large aperture which corresponds to the male pore. Each marking is transversely about 6 setal wide; the region between the markings on both sides is about 10 setal wide. Marginal edges of the quadrate area are slightly thickened and apparently weakly glandulated.

Female pore single, on XIV midventrally; (it is scarcely recognizable).

Spermathecal pores, two pairs in 5/6 and 6/7, dorsolateral in position, about 8/13 of the circumference apart. Epidermis just anteroposterior to each pore are slightly thickened and weakly wrinkled. No genital papillae are found near this region.

Internal anatomy :

No septa especially thickened; 5/6 and 6/7 slightly thickened, 8/9 ventrally traceable, 9/10 absent.

Nephridial tufts moderately thick, in V and VI.

Gizzard small, barrel-shaped. Intestine begins to swell in XV. Intestinal caeca originated in XXVII, simple, horn-shaped, small and short, extending into XXV, both ventral and dorsal margins almost smooth, but with rather distinct septal constrictions.

Hearts in IX asymmetrically developed, those in X–XIII as usual, small in calibre. Lymph glands small, found behind 15/16 caudalwards.

Testis sacs, in X and XI; anterior pair form a V-shaped sac and the posterior pair a transverse sac. Seminal vesicles large, in XI and XII, both pairs nearly equal in size, fully occupying the respective segment and dorsally meeting one another. Each with a large lobe which projects from the anterodorsal margin of the vesicle. Pseudovesicles not found. Sperm-ducts on each side meeting in XII.

Prostate gland lobular, moderate in size, in XVII–XIX. Prostatic

duct moderate in length, thin but musculated and shining, looped in an erected U-shape, its ectal part slightly thicker.

Spermathecae small, in VI and VII; ampulla elongated oval in shape; duct slender but weakly musculated, shorter than the ampulla, not sharply marked off from the latter. Diverticulum slender, nearly equal to, or a little longer than, the main portion; its ental portion slightly zigzag or simply coiled, and not markedly widened at the terminal (Fig. 6, b).

The parietes corresponding to the genital markings are slightly thickened, but not projecting into the coelom.

Remarks:

The present species is easily distinguishable from the other dithecal members of the genus found in both Korea and Japan, mainly by the characters of position of ˌspermathecal pores and of male pore aspects. It may somewhat resemble *Ph. youngi* GATES ('32), but is also distinguishable in many important characters.

The specific name of the present species was chosen as an expression of my gratitude to Prof. Dr. HARUJIRÔ KOBAYASHI.

Pheretima hilgendorfi (MICHAELSEN)

1892 *Perichaeta hilgendorfi*, MICHAELSEN, Arch. Naturg, Bd. 58, p. 235, t. 13, f. 15.

1892 *Perichaeta rokugo*, BEDDARD, Zool. Jahrb. Syst., Bd. 6, p. 756, t. 32, f. 1-7; p. 763.

1894 *Perichaeta hilgendorfi*, MICHAELSEN, Zool. Jahrb. Syst., Bd. 8, p. 192.

1895 *Perichaeta hilgendorfi*, BEDDARD, Monogr. Oligochaeta, p. 409.

1899 *Amyntas hilgendorfi*, MICHAELSEN, Mitt. Mus. Hamburg, Bd. 16, p. 9.

1900 *Pheretima hilgendorfi*, MICHAELSEN, Tierreich, p. 272.

1903 *Pheretima hilgendorfi*, MICHAELSEN, Die geograph. Verbr. Olig., p. 85.

1916 *Pheretima hilgendorfi*, MICHAELSEN, Ark. Zool., Bd. 10, No. 9, pp. 11-13.

1923 *Pheretima hilgendorfi*, MICHAELSEN, Dr. Th. Mortensen's Pacific Exp. 1914-16, XVII, p. 237.

1929 *Pheretima hilgendorfi*, HATAI, Annot. Zool. Japon., Vol. 12, No. 1, pp. 271-284.

1930 *Perichaeta hilgendorfi*, YAMAGUCHI, Zool. Mag., Vol. 42, No. 496, p. 50, figs. 1-2.

1930 *Pheretima hilgendorfi*, YAMAGUCHI, Trans. Sapporo Nat. Hist. Soc., Vol. 11, Pt. 2, pp. 89-95.

1930 *Pheretima hilgendorfi*, HATAI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. 5, No. 4, pp. 651-659.

1931 *Pheretima hilgendorfi*, YAMAGUCHI, Zool. Mag., Vol. 43, No. 511, pp. 393-399, figs. 1-2.

1934 *Pheretima hilgendorfi*, KOBAYASHI, Chosen Nat. Hist. Soc., No. 19, p. 1.

1935 *Pheretima hilgendorfi*, KOBAYASHI, Zool. Mag., Vol. 47, No. 557, p. 128.

1936 *Pheretima hilgendorfi*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 1, pp. 139 & 146.

1936 *Pheretima hilgendorfi*, KABURAKI and MISAKA, Botany and Zool., Nikkô, p. 512.

Localities:

Kankyô-nan-dô: Genzan, Tokugen, Reidôri, Eikô.

Heian-nan-dô: Chinnampo.

Kôkai-dô: Shinkei.

Kôgen-dô: Kotei, Kôjô, Kanjô, Sempo, Tetsugen, Sanchoku.

Keiki-dô: Keijô, Kôka, Monsan, Shinchiku, Rensen, Hôsen, Ryûjin, Yôshû, Anjô.

Chûsei-nan-dô: Gasan, Taiden, Zuizan.

Chûsei-hoku-dô: Seishû.

Zenra-nan-dô: Kinkai.

Zenra-hoku-dô: Zenshû, Kunsan.

Keishô-hoku-dô: Bunkei, Kinsen.

Keishô-nan-dô: Kiho, Fusan, Tôrai, Urusan.

External characteristics:

Length 85–215 mm, greatest diameter 5–9 mm; largest specimen 214 × 8.5 mm, the smallest 92 × 5.5 mm. Number of segments 92–115.

Colour in living condition, dorsally reddish brown, anteriorly and middorsally concentrated, ventrally fleshy, clitellum usually flesh-red.

First functional dorsal pore usually in 12/13; its variation is given below:

1. functional pore in 12/13.................................140 specimens
2. functional pore in 12/13, and a slight non-functional pore-like marking in 11/12.. 40   „
3. functional pore in 11/12 5   „
4. well-marked but non-functional pore in 12/13, and the next pore first functional 6   „
5. well-marked but non-functional pore in 11/12, and the next pore first functional 4   „
6. both pores in 12/13 and 13/14 well-marked but non-functional, and that in 16/17 first functional 5   „
7. pore in 12/13 slight non-functional, and the next functional.. 1   „

Setae beginning on II, moderate in size; both mid-dorsal and -ventral breaks found but slight; no marked difference in size between the post- and pre-clitellar setae; ventral setae slightly larger than, and nearly equal in interval to, the dorsal; setal number as follows: 20–30/II, 26–41/III, 35–43/IV, 39/V, 40–52/VI, 44–59/VII, 46–62/VIII, 45–71/IX, 47–65/X, 47–62/XI, 48–64/XII, 46–61/XIII, 50–66/XVII, 50–59/XX, 46–60/XXX, spermathecal setae 19–30/VI, 20–31/VII (male pore setae 17–20).

Clitellum entire, in XIV–XVI, without setae and other markings. Sometimes even in August specimens which are apparently mature, non-functional pore-like depressions on it may be found.

Male pores usually absent. Seldom found on the setal line of XVIII, being mostly unpaired, and may be found on XVI or XVII or XIX. Such abnormality of the position of the male pores was fully studied by YAMA-GUCHI ('31) on Sapporo-specimens, Hokkaido, Japan. Male porophore is a markedly prominent cone with elevated epidermis which is much wrinkl-ed; measured on a specimen picked up at random: 1.5 mm in height and 2.0 mm in base; about 1/3 of the circumference apart.

Spermathecal pores, two pairs in 6/7 and 7/8; their detailed aspects were already described by me ('37); no variation was found in the number of them (as well as in *Ph. agrestis*), at least in the present specimens examined.

Genital patch or patches are always found on VIII, or on both seg-ments VIII and IX, a single one on one segment without exception, being presetal and midventral in position, very slightly depressed, light brown in colour, nearly circular, of about 0.6-1.2 mm in diameter, transversely about $2-2 \frac{1}{2}$ setal wide; on its surface are always found several dully glistening very small papillae (its number 1-40, usually 4–18); when two patches are present on both VIII and IX, the anterior one is usually smaller in diameter and subsequently the number of papillae on it is smaller than the posterior; similar patches are also often found on XVII or XVIII, or on both of them; seldom, also on VII or X or XI, but in these cases except for the first, the outline of the patch is usually in-distinct and the number of papillae on it is much smaller; quite very seldom, no patch is found on any segment, in only three cases out of 190 examined. According to YAMAGUCHI ('30), patches found also, although very seldom, on various segments of XII–XIV and of XIX–XXI. The number of genital patches appears to be nearly constant, corresponding to each locality. Except for Keijô, I have not a sufficient number of specimens here, so such an interesting result is unfortunately unable to be shown clearly. (It may be presumable that the derivation of individuals of this peregrine form in a large town is complex comparing with that of a small village.) Following data were taken from the specimens picked up at random in the materials of several localities.

Locality	Order of segment	No. of specimens	Total
Keijô	VIII & IX	33	49
	VIII, IX, & XVIII	2	
	VIII & XVIII	2	
	VIII	11	
	absent	1	
Kotei	VIII & IX	6	8
	VIII, IX & XVIII	1	
	VII, VIII, & IX	1	
Chinnampo	VIII, IX, & XVII	9	18
	VIII, IX, XVII, & XVIII	3	
	VIII, IX, & XVIII	1	
	VIII & IX	5	
Genzan	VIII & IX	7	8
	VIII, IX, & XVIII	1	
Kinsen	VIII	7	7
Kôjô	VIII	21	22
	absent	1	
Sempo	VIII	8	8
Kunsan	VIII & IX	7	8
	VIII, IX, X, & XI	1	
Reidôri	VIII & IX	5	7
	VIII, IX, & X	1	
	VII, VIII, & IX	1	
Tôrai	VIII	13	13

Internal anatomy :

In general, septa moderately thickened; 4/5 thin, 5/6, 6/7, 11/12–13/14 moderately, 7/8, 10/11, 14/15 slightly thickened, 8/9 ventrally traceable, 9/10 absent.

Gizzard large, globular in shape. Intestine begins to swell in XV. Intestinal caeca in XXVII, complicated, each consisting of six to ten, finger-shaped secondary caeca, of which the dorsalmost is largest, extending anteriorly into about XXIII or XXIV, the more ventral ones become gradually smaller. In 125 specimens which have paired caeca, the frequency of number of secondary caeca was examined as follows:

No. of caeca	6	7	8	9	10
No. of specimens	3	26	65	29	1

Hearts in IX asymmetrical, one is larger and regular in shape and the other is usually vestigial. Those in X are usually absent (in 133 out of 190 specimens examined), but sometimes may be found as a vestigial condition (in 57 specimens). It appears interesting that most of the specimens collected from the same spot (or locality) have a common tendency of presence or absence of the rudimentary hearts in X. Hearts in XI–XIII are found as usual, but relatively small in calibre.

Testis sacs, two pairs in X and XI; anterior pair forming a rather massive V-shape and posterior pair a transverse sac. No marked variation of this organ was recognized. Testes and funnels in usual position, the former small and disc-like. Seminal vesicles small, two pairs in XI and XII, nearly equal to one another in size, dorsal lobe of the anterior pair in most cases indistinctly formed, but that of the posterior small but distinctly constricted, ovoidal and smooth on surface. Within the vesicles of about a half of the examined specimens were found Gregarin cysts in various degrees; those which are heavily infested are always much larger than those of usual size. Seminal vesicles in relative size and degree of influence of parasitism on these organs are approximately as in the following.

Size	very small (rod-shaped)	smaller	small (usual size)	moderate	large
No. of specimens	19	36	70	30	10
Degree of parasitism	always no cysts		no cyst or often with a little cysts	mostly with much cysts	with much cyst without exception

Degree of parasitism on these organ is not always parallel with that of the body as a whole. Even when the vesicles are heavily infested the testis sacs are apparently in most cases free from the cysts, and the testes appear to be healthy; in such cases the ovaries are so. Pseudovesicles, one pair, small, on posterior surface of 12/13; seldom, one more pair may be found in the succeeding segment, though rudimentary in size. Sometimes pseudovesicles are also larger than the usual size with much cysts. Sperm-ducts on each side meeting in XIII.

Ovaries moderate in size, in usual position; apparently always free from the cysts but found on parietes or gut near them.

Prostate gland and duct are totally absent when the male pores are invisible externally; in such cases sperm-duct blindly terminates in various

segments of XVII–XXX, usually behind XVIII. Showing the segmental position of the terminal end of the sperm-duct in 168 specimens, as follows:

Order of segment		17	18	19	20	21	22	23	24	25	26	27	28	29	30
No. of specimens	R	2	8	11	16	22	23	19	16	17	13	10	4	2	
	L	3	5	12	19	26	23	22	19	11	16	6	3	2	1

R right side.   L. left side.

Prostates always found when male pores present externally; each large, in about XVII-XX in most cases; duct muscular, shining on surface, usually once coiled forming an O- or U-shape, about 6 mm long if stretched, with nearly equal thickness except both slightly thinner ental and ectal ends.   The other details on this organ were previously described by YAMAGUCHI ('31) and by me ('37).

Spermathecae, two pairs in VII and VIII; ampulla elongated oval or spatulated in shape, mostly much wrinkled on surface, either with a very little content (not sexual cells) or nearly empty; duct distinctly marked off from the former, thick and muscular, shining on surface, champagne-bottle-shaped its ental portion being thinner; diverticulum always much longer than the main portion, its ectal half slender but thick-walled, often simply coiled, its ental half forming a long, thin-walled, sausage-like seminal chamber.   Seldom, very small or abnormal ones were observed.   Apparently in all cases of the spermathecae examined they were free from the cysts.

Large whitish capsulogenous glands found, corresponding to the external genital papillae on each patch.

Remarks:

The breeding habit of the present species was reported by me ('37). Considering from that result, possibly it may bread by self-fertilization (perhaps also in the other species without male pores in the genus *Pheretima*).   According to GAVRILOV ('35) (the literature of the self-fertilization in the metazoic hermaphrodites), such phenomena are not rare.   Whether it is self-fertilization or not in these *Pheretima*-species will be studied in the near future.

Recently GATES and KYAW ('37) reported an interesting examination that individuals without clitellum in *Ph. posthuma* can copulate with each other.   But, such a phenomenon is, of course, independent of the breeding habit of the groups which are annual worms and lack male or spermathecal pores.

On the kind advice of Prof. GATES, parasitism in this species was studied on the mature specimens. I much regret that from the present macroscopic study I cannot say anything decidedly on the presence or absence of the influence of parasites on the primary genitalia. But, from this result I was aware, as he especially informed me, that we must study it at first on the juvenile and immature individuals in various stages.

Pheretima kanrazana var. increta KOBAYASHI

1937 *Pheretima kanrazana* var. *increta*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 3, pp. 343–347, fig. 4.

Reisui, Zenra-nan-dô, one clitellate (its posterior part regenerated), one with incomplete clitellar glandularity, and one juvenile specimen, August, '34.

Length of the specimen with incomplete clitellar glandularity is 110 mm, number of segments 100, and that of the juvenile specimen 111. Diameter 4–5 mm. Prostomium, epilobous ca. 2/3, without posterior transverse demarcation. Colour (in formalin), uniformly whitish grey on both dorsal and ventral surfaces, clitellum light yellowish brown. First dorsal pore in 12/13, small but functional; in Mt. Kanra-specimen first dorsal pore in 12/13 was indistinct and non-functional; it may be variable in functional position. Setae on X–XIII may be a little smaller than the rest. Ventral setae are a trifle larger and slightly more closely set than the dorsal. Both dorsal and ventral breaks are present but slight or partly very slight. Setal numbers of the specimen with incomplete clitellar glandularity is as follows: 33/III, 44/VI, 53/VIII, 47/XII, 53/XVII, 49/XX, 18(VI), 19(VII), 18(VIII) between spermathecal and 15 between male pores. In clitellate specimen, spermathecal setae are 15(VI), 17(VII), 18(VIII), and male pore setae are 14.

Both spermathecal and male pore aspects are closely similar to those of the original specimens. In two specimens except the juvenile one, genital papillae on male areas are well developed and equally arranged. They consist of one postsetal and six presetal which are close together and rather regularly arranged to be transversely two by two and antero-posteriorly three by three. Male porophore is slightly sunken, forming a small circular or oval depression, into the ground of the male area, and portion situated rather far lateral of the latter. Preclitellarly no genital papillae were found as in the original specimens.

Intestinal caeca originating in XXVII, are small but complicated,

consisting of several finger-shaped secondary caeca, six on the left side and five on the right. Of the secondary caeca, the dorsalmost with very slight septal constrictions extends anteriorly into XXV, the more ventral ones become gradually shorter and smaller. Hearts in X are smaller than the rest, but not so vestigial in size as in the original specimens. Lymph glands begin to appear behind 15/16, but much more distinct behind caecal segment.

Testis sacs and seminal vesicles of the clitellate specimen are unusually large as in the original. It is much regreted that the seminal vesicles of the posterior pair have decomposed in their posterior most parts, and even their fragments were not found in any parts of the coelom. Testis sacs of the anterior pair are conspicuously large, elongated U-shape as in the original, and not so displaced posteriorwards, but extend much anteriorly as both limbs of U into about IX; anterior pair of seminal vesicles are markedly large, ship-shape elongated, ventrolaterally widely connected with the corresponding testis sac, their anterior ends extending anteriorly into about VIII, covering the greater part of gizzard dorsally and laterally also the spermathecae which are placed there. Posterior pair of testis sacs are much smaller than the anterior, but with width equal to the anterior pair ventrally and forming a quadrate sac. These sacs of both pairs are in contact with each other. In the specimen with incomplete clitellar glandularity: the anterior male organs are much smaller than those of the clitellate specimen; seminal vesicles are conspicuously large overlapping middorsally one with another, and are larger than those of the original specimen, but testis sacs are smaller than the latter. A pair of testis sacs in each segment similarly form a transverse sac, and two of both segments are closely attached with each other forming a square which is rather stout and within which sexual products are almost absent. Pseudovesicles, one pair, club-shaped, very large relatively to the body size, and on posterior face of 12/13. Accessory seminal vesicles were not found.

Prostate glands are closely similar to those of the original specimens; ducts are looped in y- or G- or inverted U-shape, its ental portion thin and ectalwards gradually increasing the thickness, and thus the ectal half is moderately thick, musculated and shining. Spermathecae are similar to those of the original specimen. But, the diverticulum is entally convoluted slightly and with a delicate sheath which was not found in the latter. A single spermatheca which was fortunately still remained in a dissected original specimen, was re-examined, but such a sheath was not

found again. In general, diverticulum may be slightly longer than the main portion if stretched.

Remarks :

Unusually large size of the anterior male organs which was previously considered to be only an individual abnormality, may not be an abnormal character, but rather may be a specific one with some variations. Group of the genital papillae on the male area appears to be constant in number. Presence or absence of the diverticular sheath is in need of further studies. As the number of specimens of both varieties *typica* and *increta* examined are few, it is still questionable to me whether or not they are specifically distinct from each other.

Pheretima levis (GOTÔ et HATAI)

1899 *Perichaeta levis*, GOTÔ and HATAI, Annot. Zool. Jap., Vol. III, p. 20, fig. 12.

Hôsen near Keijô, Keiki-dô, a single clitellate specimen, August, '35.

Description :

External characteristics :

Length 88 mm, greatest diameter 5 mm, number of segments 96. Colour in formalin, yellowish brown dorsally, lighter ventrally, clitellum chocolate. Prostomium, epilobous ca. 2/3. First dorsal pore in 12/13, distinct and functional.

Setae beginning on II, moderately large; those on preclitellar segments slightly enlarged and vaguely interrupted, those on posterior end also slightly enlarged. Ventral setae a little more closely set than the dorsal, but no marked difference in size between them. Both middorsal and -ventral breaks but slightly present. Setal number as follows: 28/III, 40/VI, 41/VII, 43/IX, 46/XX, spermathecal setae 21/VI–VIII.
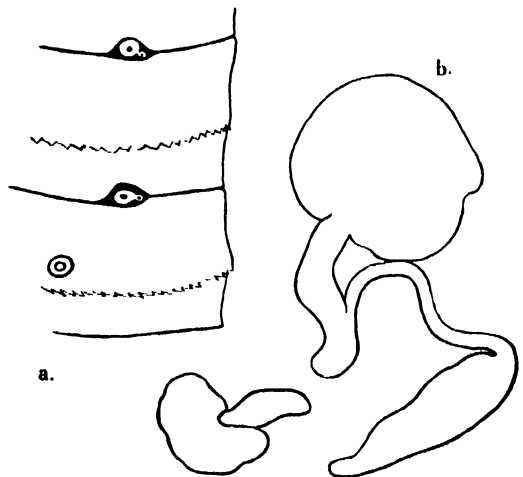
Fig. 7. *Pheretima levis* (GOTÔ et HATAI). a, ventrolateral view of spermathecal region. ×ca. 12.6 b, spermatheca with accessory gland corresponding to spermathecal papilla. ×ca. 12.6

Clitellum entire, in XIV–XVI, without setae.

Male pores absent.

Spermathecal pores, two pairs in 6/7 and 7/8, slightly smaller than 1/2 of the circumference ventrally apart. Each on a minute tip, with which just medially, always conjoined a small spermathecal papilla; these as a whole somewhat resemble a gourd, placed in intersegmental furrow not being deeply sunken (Fig. 7, a).

Genital papillae, one pair on VIII, small, circular and centrally depressed, presetal and slightly medial to spermathecal line.

Internal anatomy :

In general, no septa especially thickened; 5/6 and 6/7, and 11/12 and 12/13 slightly thickened, 7/8 and 10/11 thin, 8/9 and 9/10 absent.

Gizzard moderate in size, barrel-shaped; intestine beginning to swell in XIV; caeca in XXVII, small but complicated, each consisting of five secondary caeca, of which the dorsalmost is largest, extending anteriorly into about XXIV, the more ventral ones become gradually smaller.

Hearts in X absent; last hearts in XIII. Lymph glands small, found behind caecal segment caudalwards.

Seminal vesicles moderate in size, two pairs in XI and XII, both nearly equal in size, flattened oval in shape; dorsal lobe of the posterior pair ovoidal, and small but distinct; that of the anterior larger but indistinctly constricted.. Testis sacs, small, two pairs in X and XI; those in each segment rather widely separate from each other, those on each side closely in contact; anterior pair a little smaller than the posterior, rounded at its free anterior end, posterior pair rather somewhat quadrate in shape. Pseudovesicles, small, one pair, behind 12/13.

Ovaries moderate in size, in usual position.

Prostate gland and duct are entirely absent. Sperm-duct on each side blindly terminates, with a bulbular swelling as in *Ph. agrestis,* in XIV on left side and in XVI on right side.

Spermathecae large relatively to the body size, in VII and VIII, normal in shape in all cases (Fig. 7, b); ampulla somewhat peach-shaped being faintly pointed at its free end; duct moderate in thickness and nearly equal to the ampulla in length, sharply marked off from the latter; diverticulum always a little longer than the main portion, its ectal about 3/5 a slender but thick-walled duct, and the remaining an elongated ovoidal or sac-like thin-walled seminal chamber. Both ampulla and seminal chamber are empty in this specimen. Ampullar duct always placed to be pointing lateralwards. In VII, just anterolaterally to, and in VIII, just antero-

medially to, the ectal end of each ampullar duct, there is found a small stalked accessory gland which communicates with the spermathecal papilla. So, the glandular portions of these glands are placed both medial and lateral to the spermathecal line.

Corresponding to the external genital papillae, slightly larger but similar accessory glands are found in VIII; these are placed slightly medially and posteriorly to the former glands of the posterior pair. These three glands on each side are placed at nearly equal distance from one another.

Remarks :

Although its original description is brief, the present specimen closely resembles it in many characters. In view of rather wide intraspecific variation, the presence or absence of genital papillae and difference in number of spermathecal papillae may be of little significance in this case. Degree of dilatation of the spermathecal ampulla is, however in a small degree, also variable owing to the amount of the content within it. Thus, I think the present specimen is identical with *Ph. levis* (GOTÔ et HATAI).

MICHAELSEN classified the present species as a synonym of *Ph. hilgendorfi* in his Tierreich. But, from the present description it is clear that they are specifically distinct from each other. As already stated, it resembles in many characters *Ph. irregularis* and *Ph. koreana*.

Pheretima soulensis, n. sp.

Kôryo, Keiki-dô, three clitellate, 7 aclitellate, and 1 juvenile specimens, September, '35.

Keijô, 2 clitellate specimens, July, '36.

Description :

External characteristics :

Length 51–86 mm, greatest diameter up to 5 mm, number of segments 83–95. Colour in formalin, yellowish to reddish brown dorsally and concentrated middorsally. much lighter ventrally, clitellum chocolate or russet. Prostomium, epilobous ca. 1/2. First dorsal pore in 12/13. Clitellum entire, in XIV–XVI, without setae.

Setae beginning on II, moderate in size; preclitellar setae may be rather smaller than the postclitellar; usually those on posterior segments enlarged and planted on slightly elevated ridges. Mid-dorsal and -ventral breaks found but slight. Setal number as follows: 28–30/III, 39–44/VI, 41–47/VII, 44–52/IX, 44–45/XX, 46–53/XXX, spermathecal setae 18–19/

VI, 19–21/VII, 20–23/VIII, male setae 15–16.

Externally it appears that the male pores are present as in the normal species, but internally no prostate and no duct are found, and the sperm-duct blindly terminates as in *Ph. agrestis*.

Ventral aspects of XVIII are slightly different between Kôryô- and
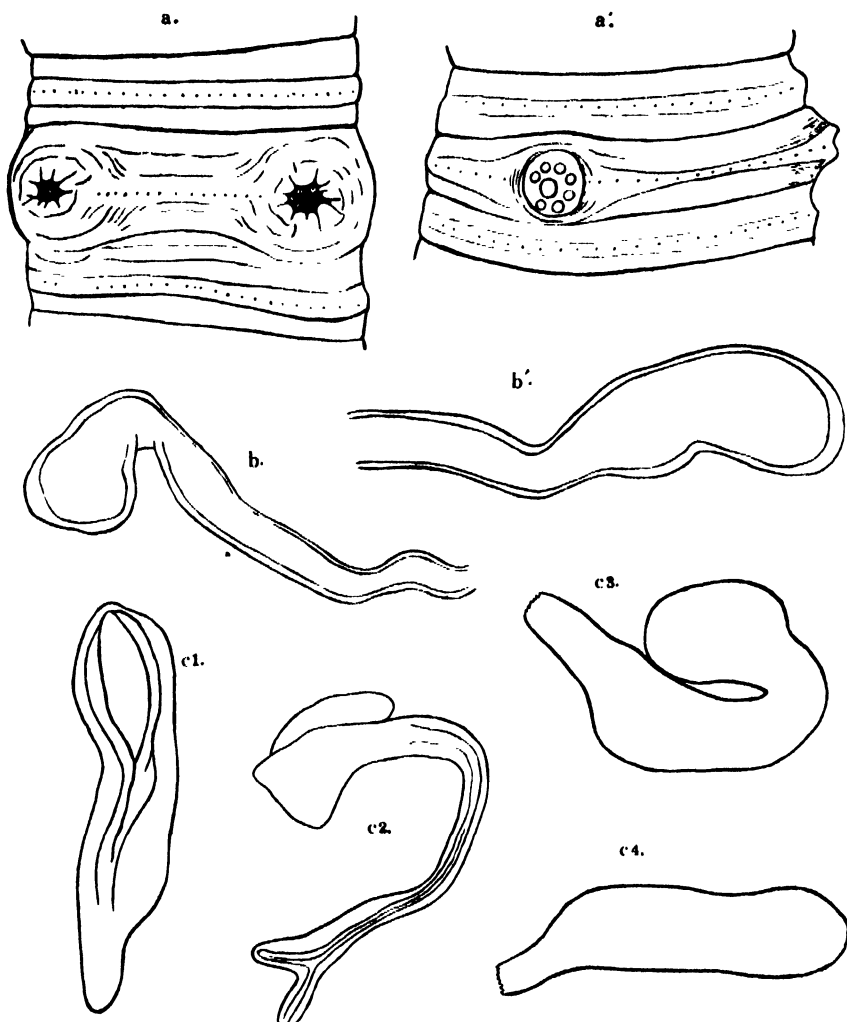
Fig. 8. *Pheretima soulensis*, n. sp. a & a′, ventral or ventrolateral view of XVII–XIX. ×ca. 12.6 (a, Keijô-specimen, and a′, Kôryô-specimen). b & b′, terminal ends of sperm-ducts. ×ca. 48.5   c1–c4, spermathecae all vestigial in size. ×ca. 12.6 (3–4 not cleared).

Keijô-specimens, but of course no constituent difference was found; it might be caused by the different degree of the contraction of the worms when they were killed. Small male (?) discs are moderately protruded. (1) (Fig. 8, a'), those of Kôryô-specimens are all wholly or almost wholly everted, while (2) (Fig. 8, a), those of Keijô-specimens are moderately withdrawn into the body cavity and concealed by the glandulated and thickened epidermis around them. (1) Aspects of the discs somewhat resemble B-type of *Ph. hesperidum* (=*Ph. californica*) which were illustrated by CHEN ('33). Discs are situated on the setal line of XVIII, slightly less than 1/2 of the circumference apart, small but prominent; each disc occupying about middle 3/5 of the anteroposterior length of the segment at its base. On top of the disc which is circular and nearly flat, is found a small papilla (resembles male porophore in the normal species of the genus) at the centre, and also found is around the former three to six much smaller dully glistening genital papillae; these porophore-like body and genital papillae are all sunken into the ground of the disc; so, their appearance as a whole somewhat resembles a dial of telephone or the surface of a lotus fruit (if porophore-like body is similar in size to the genital papillae). Setal zones, between both discs, and also of the dorso-lateral sides are distinctly elevated from the general surface. (2) Secondary male pore-like opening represented as a large aperture with much marginal ridges on moderately-sized epidermal conical elevation which is extending anteriorly near to 17/18 and posteriorly slightly over beyond 18/19. Within this opening four to five genital papillae is found around a much larger but indistinctly elevated body.

Spermathecal pores, two pairs in 6/7 and 7/8, about 4/11 of the circumference ventrally apart; epidermis around each pore slightly wrinkled and more deeply pigmented than the rest.

Internal anatomy :

Septa, in general, slightly thickened; 5/6 and 6/7 moderately, 10/11–12/13 slightly thickened, 7/8 thin, 8/9 and 9/10 absent.

Gizzard large, bell-shaped; intestine begins to swell in XV. Intestinal caeca in XXVII, complicated, each consisting of five or six finger-shaped secondary caeca as in *Ph. hilgendorfi*, of which the dorsalmost is longest, extending anteriorly into about XXIII, the more ventral ones become gradually shorter.

Hearts in X not found, the others as usual. Lymph glands small, found behind caecal segment caudalwards.

Seminal vesicles, two pairs in XI and XII, moderate in size and nearly

equal to each other; each with a distinctly constricted, relatively large, ovoidal lobe. Testis sacs small, two pairs in X and XI; anterior pair ovoidal, scarcely connected and communicated at the posterior end with each other by a short and thin bridge forming a V-shape; posterior pair broadly united, forming a quadrate sac, but a faint, longitudinal depression is found along its median line on both dorsal and ventral surfaces. Pseudo-vesicles not found.

Prostate gland and duct are entirely absent. The region internal to each disc is slightly thickened but not projecting into the coelom, and there are only found three to six, rather large, whitish capsulogenous glands corresponding to the external genital papillae. Sperm-ducts blindly terminate in all cases with a bulbular or spatulated minute swelling (Fig. 8, b & b'). In two Kôryô-specimens, those on both sides terminate just on these thickenings, but are quite free from the parietes; in one Keijô-specimen examined they similarly blindly terminate in XVII on left side and in XVI on right side.

Spermathecae in VII and VIII, quite vestigial in size (Fig. 8, c_1–c_4); diverticulum not found in all cases examined. Ampulla of the second pair is only very slightly dilated, but that of the first pair is apparently not distinct from the duct portion, though it is distinguishable from the latter under the microscope. In right side of the second pair of one Keijô-specimen was found, as the only case, a large spermatheca with diverticular portion but quite vestigial in size and also in structure (Fig. 8, c_4).

Remarks:

In spite of that the genital markings on XVIII, they are quite similar in appearance to those of the normal species of the genus, no male pores were found, and internally the sperm-ducts blindly terminate.

The present species is very close to *Ph. shinkeiensis* in many respects, but clearly differs in the ventral aspects of XVIII. The characteristic appearance of the disc, and secondary male pore-like opening are quite different from each other, i. e. if male pores are present in both species, it must be of invaginate in the present species, and of a epidermal in the latter species.

Pheretima shinkeiensis, n. sp.

Shinkei, Kôkai-dô, a single clitellate specimen, August, '35.
Description:
External characteristics:

Although the exact measurements for body length and number of segments of this specimen could not be made, it may be said to be a small-sized worm; diameter about 5 mm in XI. Colour in formalin, dark grey dorsally and somewhat concentrated middorsally, pale grey ventrally; clitellum flesh brown. Prostomium, epilobous ca. 1/2. First dorsal pore in 12/13.

Setae beginning on II, moderate in size; middorsal break distinct, but midventral one rather indistinct. Ventral setae a little more closely set than the dorsal; nearly equal in size between them. Setal number as follows: 31/III, 44/V, 43/VI, 46/VII, 52/IX, 49/X, 53/XX, spermathecal setae 17/VI, 18/VII, 19/VIII.

Clitellum entire, in XIV–XVI, without setae.

Male pores were not found. The ventro-lateral walls of XVII–XIX are thickened and whitened, and the lateral part of XVIII is slightly stretched lateralwards. In such condition, three very small genital papillae are found close together on the setal line of and on the ventrolateral wall of XVIII. I thought at first that the male pores are to be found as usual near or between these papillae, since the appearance of XVIII is quite similar to the other normal species of the genus. But, no pores were found anywhere under the low power microscope. Between these papillae on both sides were found 16 setae, and they are about 1/3 of the circumference ventrally apart (Fig. 9, a).

Fig. 9. *Pheretima shinkeiensis*, n. sp. a, ventral view of XVII & XVIII. ×ca. 12.6   b. spermatheca without both ampulla and diverticulum. ×ca. 28

Female pore single, midventrally on XIV.

Spermathecal pores, two pairs in 6/7 and 7/8, ventrolaterally, about 5/13 of the circumference apart. Each pore is of a simple opening in intersegmental furrow. On both anterior and posterior borders of each pore are found slightly wrinkled, pigmented in light-orange (in formalin), and indistinctly demarcated areas which are not depressed from the general

surface and without any structures as in *Ph. agrestis.*

There are no more genital marking elsewhere.

Internal anatomy :

In general, no septa especially thickened ; 5/6, 6/7, 11/12–13/14 slightly thickened, 7/8 thin, 8/9 ventrally quite vestigial, 9/10 absent.

Gizzard small ; intestine beginning to swell in XV ; caeca in XXVII, small but complicated, each consisting of six secondary caeca, of which the dorsalmost is largest, extending anteriorly into a part of XXIV, the more ventral ones become gradually smaller.

Hearts in X not found ; last hearts in XIII. Lymph glands small, found behind caecal segment caudalwards.

Seminal vesicles, two pairs, in XI and XII, very large relatively to the body size, fully occupying the respective segment, each with a large dorsal lobe. Each vesicle is heavily parasitised by Gregarin cysts ; their very large-size may be reduced in part to the influence of parasitism. Testis sacs relatively small, two pairs in X and XI, each pair forming a ventral single sac, and both are in contact with each other. Pseudovesicles not found.

Prostate gland and duct are entirely absent. Sperm-duct on each side blindly terminates as in *Ph. hilgendorfi,* etc.; on left side in middle part of XV, and on right side in anterior part of XV. In XVIII, three very small whitish capsulogenous glands are found corresponding in position to the external genital papillae.

Ovaries rather large, in usual position.

Spermathecae in VII and VIII. They are all vestigial in size ; each has no ampulla and no diverticulum, but only a short and small but muscular duct (Fig. 9, b).

Remarks :

I had opportunities to examine several species without male pores in this genus, such as *Ph. hilgendorfi, Ph. agrestis, Ph. vittata, Ph. koreana, Ph. irregularis,* and *Ph. levis ;* and on the first three species, a number of specimens collected from many localities were examined (specimens with male pores were rarely found). Of these three, in *Ph. hilgendorfi* possess some genital patch on male segment without male pores, but always midventrally. In *Ph. agrestis* when genital papillae are present on XVIII, male pores are always found near the former. In *Ph. vittata* even when genital papillae are absent on XVIII, male pores are frequently found.

In the present species as well as in *Ph. soulensis,* from the status of

XVIII male pores appear much likely to be found, but no pores were found near the genital papillae, and internally the sperm-ducts blindly terminate as in *Ph. agrestis*. These two species appear to be an intermediate form respectively between the forms with and without male pores.

Pheretima masatakae (BEDDARD)

1937 *Pheretima masatakae*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 3, pp. 337–340, fig. 2.

Kwantô, a small island slightly apart from the peninsula, Zenra-nan-dô, one clitellate and eight aclitellate specimens, August, '35.

Mokpo, Zenra-nan-dô, a single clitellate specimen (its posterior part incomplete), August, '35.

Nankai, a small island, Keishô-nan-dô, a single clitellate specimen, August, '35.

Chintô, a small island near Mokpo, three clitellate and one aclitellate specimens, March, '34.

Sansei, Keishô-nan-dô, a single clitellate specimen, August, '35.

Description:

Length 102–130 mm, greatest diameter 5.5–7 mm, number of segments 114–130. Colour, dark brown and concentrated middorsally and preclitellarly, ventrally lighter, clitellum reddish brown or reddish orange. First dorsal pore is invariably in 11/12, distinct and functional. Setal arrangement is quite similar to that of the Quelpart Island-specimens. Setal number as follows:

Locality	III	IV	VII	VIII	IX	XII	XX	VIIS	VIIIS	IXS	MP
Chintô	20	28	39	41	44	47	56	13	14	15	14
Kwantô	26	36	45	49	48	57	64	16	16	17	13
	27	32	44	45	45	47	58	15	16	17	15
Mokpo	24	32	40	40	44	48	56	15	15	15	14
Nankai	30	37	43	50	52	54	54	14	15	15	15
Sansei	24	31	40	42	43	48	56	15	16	16	14

Male and spermathecal aspects are quite similar to those of the Q. Is.-specimens, but, in general, the male area as a whole is a little more elevated than that of the latter, forming a rather large disc, and the ventral surface of that segment is slightly thickened. Genital papillae on both spermathecal and male pore regions closely resemble those of the

latter in arrangement and also in occurrence; but, preclitellarly the additional ones may be found in the same line either on posteriormost border of IX or on both posteriormost border of IX and anteriormost border of X facing each other; in an aclitellate Kwantô-specimen an additional pair is found presetally on XIX in line with those of XVIII. Even in the aclitellate specimens, genital papillae on male area are rather well-developed, but those near the spermathecal pores are poorly developed. One, or less frequently two, setae were found on each male area.

Intestine begins to swell in XV. Intestinal caeca simple, finger-shaped, without indentations but with slight septal constrictions, originating in XXVI, extending anteriorly into XXIII or seldom into XX. Hearts in IX asymmetrical, those in X–XIII as usual, dorsal vessel conspicuously enlarged in region of about X–XV. Lymph glands small or moderate in size, found behind 15/16 caudalwards, and may be a little larger in the posterior part of the body.

Seminal vesicles are always very small, oval or rather strap-shaped, each with a relatively large or very large dorsal lobe, sometimes this lobe may be subdivided into two or three smaller ones. Testis sacs small; anterior pair forming ventrally a low V-shaped sac and posterior pair a transverse sac. Pseudovesicles small, one pair. Prostatic glandular portion is usually absent. In three specimens, the largest specimen of Kwantô, a Chintô-specimen, and a Mokpo-specimen, the quite rudimentary glandular portion was found on one side only respectively; that of the Mokpo-specimen is differrent from the others, i. e. it is discoidal in shape, stout, and sessile on parietes or rather slightly deposited into the body wall, somewhat resembling that of some *Drawidian* prostate. Ducts are always present but very small, bow-shaped, thin but musculated and shining. Accessory glands near this region are slightly smaller than those of the spermathecal region. Spermathecae of all specimens at hand closely resemble one another and are similar to those of the Q. Is.-specimens. In spite of the smaller size of the ampulla, the duct is very thick and musculated. Ental end of the diverticulum is always well distended, forming a large seminal chamber.

Remarks :

On Chintô-specimens, I have already made mention in the appendicular note of my previous paper ('37). Localities of the present specimens are restricted to the south-western coast of the peninsula where various communications and business is carried on with the towns in Quelpart Island, and no specimens have been collected from " inland ".

In general, all characters except the body size and rare presence of the prostatic glandular portion are rather constant. Its male and spermathecal aspects are characteristic and constant, so only by which it is easily distinguishable from the other Korean species of the genus.

In MICHAELSEN's paper ('03), it was noted that the original locality of the present species is Yokohama, Japan; but, so far as I am aware, no record was given in the original description by BEDDARD. This locality might be noted by BEDDARD on the type-specimens.

Pheretima reisuiensis, n. sp.

Reisui, Zenra-nan-dô, a single clitellate (but, its clitellar glandularity not yet complete) specimen, August, '34.

Description:

External characteristics:

Length 91 mm, greatest diameter 4.5 mm, number of segments 105. Colour faded. Prostomium, epilobous ca. 2/3. First dorsal pore in 11/12, distinct and functional. Clitellum entire, in XIV–XVI; but, its clitellar glandularity not yet complete being not obliterate intersegmental furrows and setal pits.

Setae beginning on II, moderately large; both mid-dorsal and -ventral breaks present but very slight. Setae on III–IX and on several segments of the posterior end of the body enlarged. Ventral setae slightly larger than the dorsal, but in interval no marked difference is found between them. Setal number as follows: 25/III, 35/VI, 43/IX, 57/XX, 60/middle portion of the body, spermathecal setae 14/VII, 15/VIII, 15/IX, male pore setae 10; 3–4 between spermathecal pore and genital papilla on each side.

Male pores on the setal line of XVIII, about 1/3 of the circumference ventrally apart. Secondary male pore represented as a large eye-like aperture; the region around the aperture glandulated, whitened, and slightly elevated, extending a little beyond 17/18 and 18/19 into both anterior and posterior segments. Within each secondary male pore, a minute spherical porophore is found externally. No genital papillae found near the pores. (Fig. 10, a).

Female pore single, midventrally on XIV.

Spermathecal pores, two pairs in 7/8 and 8/9, about 1/3 of the circumference ventrally apart. Just posteriorly to each pore is found a very small ovoidal spermathecal papilla which is slightly sunken into the intersegmental furrow. Genital papillae found presetally on both segments
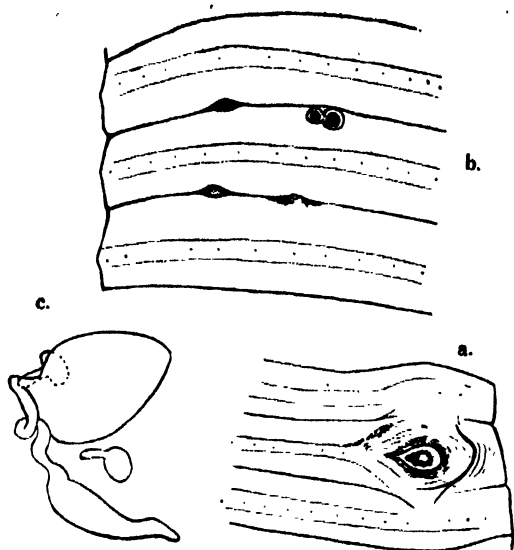
Fig. 10. *Pheretima reisuiensis*, n. sp. a, ventro-
lateral view of male pore region, ×ca. 12.6. b,
ventrolateral view of spermathecal region, ×ca.
12.6. c, spermatheca with accessory glands corre-
sponding to both genital and spermathecal papillae,
×ca. 12.6.

of VIII and IX; placed medially with 3–4 setae to the spermathecal pore and close to the intersegmental furrow; circular with slight central depression and a little larger than the spermathecal papillae. Only on right side of VIII, are found two papillae which are conjoined transversely with each other (Fig. 10, b).

Internal anatomy:

Septa moderately thickened; 5/6, 6/7, 10/11, and 11/12 much or moderately thickened, 8/9 and 9/10 absent.

Gizzard small, globular; intestine beginning to swell in XV; caeca simple, finger-shaped, smooth on both margins, but with rather distinct septal constrictions, situated in XXVII–XXII.

Hearts in IX asymmetrically developed, those in X–XIII as usual, and small in calibre. Lymph glands not found.

Testis sacs small, two pairs in X and XI; anterior pair ventrally forming a low V-shape, and posterior pair a transverse sac; these two sacs of both segments are distinctly separated by a thick septum 10/11. Seminal vesicles, two pairs in XI and XII, small or rather strap-shaped, each with a relatively large dorsal lobe. Pseudovesicles, one pair, relatively large, on posterior face of 12/13.

Prostate gland moderate in size, in XVII–XIX, somewhat quadrate in shape; duct moderate in length, thin but musculated, *l-* or *u*-shaped, both ectal and ental ends much thinner than the rest. Ectal end of the duct opens directly on to the exterior through the male porophore.

Spermathecae very small; ampulla peach-shaped, its duct very short but relatively thick and musculated, not sharply marked off from the former; diverticulum always longer than the main portion, its ectal half

rather slender and simply twisted, and the ental remaining slightly dilated and forming a flame-shaped seminal chamber. Near each spermatheca, and just behind the septum, a very small stalked accessory gland is found (in right side of VIII two glands) corresponding to the external genital papillae; and just close to the spermathecal duct a similar gland with short stalk is found corresponding to the spermathecal papilla. (Fig. 10, c).

Remarks:

The present species resembles *Ph. masatakae*, *Ph. robusta*, and *Ph. schmardae.* But, it differs from these three mainly in both male and spermathecal aspects, and also, from the first in presence or absence of lymph gland and in shape of spermathecae, from the second in the structure of the anterior male organs, and from the third in shape of the intestinal caeca.

Pheretima agrestis (GOTO et HATAI)

1899 *Perichaeta agrestis*, GOTÔ and HATAI, Annot. Zool. Jap., Vol. III, p. 17, fig. 17.
1900 *Amyntas agrestis*, BEDDARD, Proc. Zool. Soc., London, p. 609.
1900 *Pheretima agrestis*, MICHAELSEN, Tierreich, pp. 313 & 272.
1930 *Perichaeta agrestis*, YAMAGUCHI, Zool. Mag., Vol. 42, No. 496, p. 51, fig. 3.
1930 *Pheretima agrestis*, HATAI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. 5, No. 4, pp. 651-655, fig. 1.
1935 *Pheretima agrestis*, KOBAYASHI, Zool. Mag., Vol. 47, No. 557, p. 128.

Localities:

Kankyô-nan-dô: Genzan, Kankô.
Heian-nan-dô: Chinnampo.
Keiki-dô: Keijô, Ryûjin, Anjô, Heiten.
Kôgen-dô: Sempo.
Chûsei-nan-dô: Taiden, Gasan.
Zenra-hoku-dô: Zenshû, Kunsan.
Zenra-nan-dô: Reisui, Mokpo.
Keishô-nan-dô: Fusan, Urusan.

Description:

External characteristics:

Length 86–158 mm, greatest diameter 5–8.5 mm; largest specimen 156 × 8 mm, and the smallest 86 × 5 mm. Number of segments 91–110 (from 121 specimens), usually 100–107. In general, slightly smaller than *Ph. hilgendorfi.* Colour almost similar to that of the latter species, but clitellum in the present species in most cases fleshy orange.

First functional dorsal pore usually in 12/13, but considerably variable.

1. functional first dorsal pore in 12/13.....................133 specimens .
2. pore in 12/13 well-marked but non-functional, and the next first functional... 30
3. both pores in 12/13 and 13/14 well-marked but non-functional, and that in 16/17 first functional 23
4. pore in 12/13 functional, and a slight non-functional pore-like marking in 11/12 11
5. each pore in 12/13, 13/14, and 16/17 well-marked but non-functional, and that in 17/18 first functional 9
6. adding to the case (5), a slight non-functional pore-like marking in 11/12 4
7. pore in 12/13 slight, non-functional pore-like marking, and the next functional 3
8. both pores in 11/12 and 12/13 slight, non-functional pore-like marking, and the next functional 3
9. each pore in 11/12, 12/13, and 13/14 slight non-functional pore-like marking, and that in 16/17 functional........... 1
10. pore in 11/12 well-marked but non-functional, and the next functional 1
11. both pores in 12/13 and 13/14 slight non-functional pore-like marking, and that in 16/17 first functional........... 1

Setae beginning on II, moderate in size; mid-dorsal and -ventral breaks present but slight, and sometimes indistinguishable. No marked difference in size between the preclitellar and postclitellar, and also between the ventral and dorsal, but the lateral ones are usually smaller than both ventral and dorsal throughout the entire length of the body; ventral setae slightly more closely set than the dorsal. Setae on each genital patch invisible, but sometimes their rudiments or setal pits were recognizable even in the clitellate specimens. Setal number as follows: 24–37/II, 28–44/III, 32–50/IV, 38–50/V, 42–54/VI, 46-58/VIII, 47–63/IX, 48–67/X, 50-66/XI, 53–64/XII, 53–65/XIII, 51–64/XVII, 51–66/XX, 50–59/XXX, spermathecal setae 18–25/V. 20–29/VI, male pore setae 5 (only one specimen with paired male pores; in the other specimen if the male pores are pairly present, possibly about 8 setae may be counted between them).

Clitellum entire, in XIV–XVI, without setae; but, sometimes, even in August-specimens which are apparently mature, non-functional pore-like markings may be found on it.

Male pores always absent. But, two were found out of two hundred and forty-eight specimens; one has a pair of pores, and the other has a single pore on the right side only. Secondary male pores situated ventro-laterally on the setal line of XVIII, about 1/3 of the circumference apart; each represented as a rather large, transverse, slit-like invagination (or

slightly diagonal in the other specimen, with its medial side posterior), placed on a circular, glandulated but not protruded, indistinctly outlined genital area which is close with its posterior border to the intersegmental furrow 18/19 and is apart by about 1/2 of the presetal length of XVIII from 17/18; primary male pore invisible externally. A pair of conspicuously large, transversely oval, markedly protruded but flat and smooth on surface, light-coloured genital papillae situated presetally on XVIII being very close midventrally with each other and also very close laterally to each male genital area; posterior border of each papilla close to, or rather interrupting, the setal line of XVIII, and its anterior margin extending a trifle beyond 17/18 into XVII; its transverse length about 1.8 mm, and the anteroposterior 1.5 mm. Presetal length of XVIII longer than the postsetal, and the total length of the segment longer than the rest of the postclitellar; between male pores present five setae, but no setae found between male pore and genital papilla on each side. In the specimen with a single male pore, a similar papilla was found on the same side where the pore is placed.

Female pore single, midventrally on XIV.

Spermathecal pores, three pairs in 5/6, 6/7, and 7/8, about 1/3 of the circumference apart, each rather large, transversely slit-like, on the minute oval tubercle which is sunken into the intersegmental furrow. No variation in number of spermathecal pores was found, similarly to *Ph. hilgendorfi*.

Two or three pairs of genital patches usually found on both sides of the midventral line of VII and VIII, or of VI, VII, and VIII; each patch light brown in colour, not protruded but slightly glandulated (in the preserved specimens, delicately wrinkled on surface), almost squarish in shape but without distinct demarcation, transversely about five to ten setal wide, longitudinally not extending over a whole segment, a small part remaining on both sides anteriorly and posteriorly; a pair on each segment separated by 0–6 setae, and about 2–6 setae found between patch and spermathecal pore on each side; no setae found on each patch, but sometimes, their rudiments or setal pits may be found, and in general, in younger specimens normal setae are clearly visible on each of them. No genital papillae found on the patch. Patches are considerably variable as to number, form, and situation; those cases are approximately included within those illustrated by HATAI ('30).

Internal anatomy:

Septal thickness quite similar to that of *Ph. hilgendorfi.*

Gizzard large, globular. Intestine begins to swell in XV. Intestinal caeca in XXVII, complicated as in *Ph. hilgendorfi*, but secondary caeca are of five to eight pairs; showing the frequency of number of secondary caeca examined in the specimens with paired ones as follows: five pairs in 38 specimens, six pairs in 63, seven pairs in 69, and eight pairs in 16.

Vascular system apparently similar to that of *Ph. hilgendorfi*.

Seminal vesicles, two pairs in XI und XII, relatively large, almost meeting middorsally with one another, each with a dorsal lobe, which is not distinctly constricted in the anterior pair, and is slightly larger and in most cases distinctly constricted in the posterior pair. Testis sacs moderate in size, two pairs in X and XI; anterior pair ventrally forming a low V- or U-shape and the posterior a transverse sac; these are in contact with each other. Testes small, situated in usual position. Sperm-ducts on each side meeting in XIII. Pseudovesicles, one pair, small or sometimes relatively large, behind 12/13; seldom, one more pair may be found in the succeeding segment, though much smaller.

Ovaries moderate in size, in usual position.

Prostate gland and duct are totally absent. Sperm-duct blindly terminating with a bulbular swelling in various segment of XIV–XXI; showing the frequency of terminal segment of the sperm-duct as follows:

Order of segment	.	14	15	16	17	18	19	20	21
No. of specimens	R	2	32	49	52	28	7	5	2
	L	0	37	58	55	21	5	2	0

As is clear from this result, they usually terminate in front of XVIII, and seldom, behind it. Prostate is found in two specimens which have male pores externally. Gland large, meeting middorsally with its opposite if present, in XV- or XVI–XXII, each consisting of three or four main lobes. Duct rather thick, moderate in length, about 5 mm long if stretched, muscular, shining on surface, looped in a hair-pin-shape with nearly equal thickness, but its ectalmost is much thinner than the rest and enters into a small, round, light-coloured, softened, cushion-like glandular mass (about of 0.9 mm in diameter) which may possibly be protrusible externally. Very closely and anteromedially to these glandular masses, or just laterally on both sides of the ventral nerve cord, two whitish large softened semi-spherical accessory glands are markedly protruded · into the coelom of XVIII.

Spermathecae, three pairs in VI, VII, and VIII, moderate in size. Ampulla dorsoventrally flattened oval, irregularly much wrinkled on surface, sometimes also marginally zigzaged, in most cases either empty or with a very little content; duct thick, muscular, shining on surface, distinctly marked off from the former, its ental portion slightly thicker than the ectal;. diverticulum always longer than the main portion, its ectal half slender but rather thick-walled, the ental half forming a long thin-walled sausage-like seminal chamber which usually contains a whitish mass.

No accessory glands of any kind are found inside the region corresponding to the external genital patches.

Remarks :

Unfortunately I was unable to examine the influence of parasites on the genitalia in this species, since I received the information on this theme from Prof. GATES after the present description was almost accomplished. But, the present species is also heavily infested by parasites in many specimens as in *Ph. hilgendorfi.*

The present species was classified by MICHAELSEN in his Tierreich as a variety of *Ph. hilgendorfi.* Of course, they closely resemble each other in many respects; but, from HATAI's re-examination ('30) and also from the present descriptions on both species it is clear that they are specifically distinct from each other.

Specimens with male pores in the present species are very rare. Ventral aspect of the male pore region resembles that of *Ph. hataii* OH-FUCHI but some differences are found in the other characters.

Appendicular note on **Pheretima agrestis** (GOTO et HATAI).

Keijô, two clitellate specimens, September, '34.

During the last four years, hundreds of worms were collected and examined by myself, and this species can be identified at a glance without making any careful examination of the body, from its general appearance and behaviour, especially from its characteristic genital patches which are found on spermathecal region. The specimens in question were collected from near the place where the typical form is abunduntly found. They are different from the latter in the following points. (1) colour in living condition, much more dusty. (2) rather sluggish in locomotion. (3) both specimens lack the genital patches. (4) setae small or rather delicate partly. (5) setal number on the postclitellar segments is more numerous; 70 and 72 on XX, while in 219 specimens of the typical form it ranges

from 51–66 on the same segment. (6) shape of spermathecae; ampulla is not so wrinkled on surface, and duct is not so sharply marked off from the former, as in the latter form; diverticulum is nearly equal in length to the main portion, but in the latter form it is always much longer; seminal chamber is not sausage-like, but only elongated oval. But, since in many other respects these are quite similar to the typical form, they must be specifically identical with *Ph. agrestis*.

When we have but few specimens belonging to both of *Ph. agrestis* and *Ph. hilgendorfi* which equally lack the genital patches and male pores, and as they are very close in many respects, specific distinction becomes much difficult. But, we can distinguish one from the other if the statistical results on the important characters of both species are provided with, in (1) spermathecal number, (2) general form of the body, (3) number of secondary caeca, (4) segmental position of the terminal end of sperm-duct, and (5) size of seminal vesicles.

Pheretima phaselus HATAI

1930 *Pheretima phaselus*, HATAI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. V, No. 4, pp. 659-661, fig. 6.

Keijô, two clitellate specimens, but their clitellar glandularity is not yet complete, August, '35.

The present specimens are poorly preserved and already macerated, so it was difficult to examine in detail the structures. The characteristic kidney-bean-shaped male patches, shape of spermathecae, approximate setal number, body size, and clitellar colouration closely resemble those in the original description of *Ph. phaselus*. Its full description will be reported with the accumulation of fresh materials.

Ph. phaselus is very close to *Ph. kamitai*, and perhaps differs only in the aspects of the male pore. *Ph. kamitai* may be a special form of the present species if the testis sacs are similarly annular in shape. Testis sacs of the latter species were not described by HATAI in his original paper, and those of the present specimens have been unfortunately destroyed.

Pheretima kamitai KOBAYASHI

1934 *Pheretima kamitai*, KOBAYASHI, Jour. Chosen Nat. Hist., No. 19, pp. 5 6.
1936 *Pheretima serrata*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol XI, No. 1, pp. 165 168.

Kôjô, Kôgen-dô, a single clitellate specimen, August, '35.

Gasan, Chûsei-nan-dô, a single clitellate specimen, August, '35.

Seishû, Chûsei-hoku-dô, a single clitellate and two juvenile specimens, August, '36.

Kotei, Kôgen-dô, two clitellate and several aclitellate and juvenile specimens, August, '35.

Description :

External characteristics :

Length 82-129 mm, greatest diameter up to 5.5 mm, number of segments 100-118. Colour in formalin, reddish brown dorsally and slightly concentrated anteriorly and middorsally, light brown to pale ventrally, light chocolate or russet in clitellum. First dorsal pore in 12/13, distinct and functional; in Gasan-specimen, pore in 11/12 non-functional pore-like marking, those in 12/13 and 13/14 distinct but non-functional and that in 16/17 first functional. Clitellum entire, in XIV-XVI, without setae.

Setae small or rather moderate in size, and may be slightly enlarged and irregularly interrupted on about III-IX; ventral setae slightly larger and a little more closely set than the dorsal. Both mid-dorsal and -ventral breaks very slight if present. Setal number as follows :

Locality	III	V	VI	IX	XX	SV	SVI	SVII	SVIII	MP
Gasan	34	45	57	62	67	16	17	18	18	14
Seishû	33	50	55	54	62	16	17	17	17	14
Kôjô	30	44	46	51	59	12	12	14	16	11
Kotei 1	36	49	51	54	63	15	16	16	16	12
Kotei 2	34	51	51	55	64	14	16	16	17	14

Male pore situated on small circular or transversely oval, moderately elevated papilla which is of about 0.4-0.6 mm in diameter, and occupies about 3/5 (sometimes 1/3) of the antero-posterior length of XVIII. Surface of this papilla is nearly flat or rather slightly concaved, and the pore is opened with a trifle elevation on the lateral part of it (Fig. 11). But, this situation of the pore may sometimes displace to be near to the centre of the papilla. The pores are about 2/7 of the circumference ventrally apart.

Spermathecal pores, three pairs in 5/6-7/8, about 2/7-3/10 of the
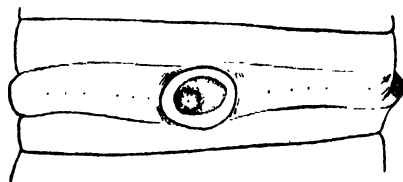
Fig. 11. *Pheretima kamitai* KOBAYASHI. Ventrolateral view of XVIII, ×ca 12.6.

circumference ventrally apart; each on a small tip which is either slightly sunken into the intersegmental furrow or slightly elevated from the remaining portion around it.

Internal anatomy:

Septa generally much thickened; 5/6, 6/7 very much, 7/8 considerably, 8/9 absent or may be ventrally traceable, 9/10 absent, 10/11–12/13 also considerably, 13/14 slightly thickened.

Intestinal caeca simple, horn-shaped, in XXVII, each ventrally or sometimes also dorsally but proximally only with several serriformed outgrowths, and with rather distinct septal constrictions, extending into about XXIV.

Hearts X and XI are enclosed within the respective annular testis sacs. Lymph glands large, whitish, lobular, found behind caecal segment caudalwards; seldom, inconspicuous ones may be found in front of the region.

Both testis sacs in X and XI are annular or seldom vertical U-shape (Gasan-specimen), encircled the gut, and enclosed within them the dorsal and ventral vessels, and hearts, and within that of XI also the respective seminal vesicles. Seminal vesicles small or very small, each with a small but distinctly constricted dorsal lobe. Pseudovesicles, one pair, very small, found behind 12/13, sometimes absent.

Prostate glands large, somewhat quadrate in shape, in XVI- or XVII–XX or –XXI. Duct relatively short and small to the large size of the glandular portion, looped in C- or U-shape, with nearly equal thickness, or with the terminal portion only very slightly enlarged.

Ovaries relatively large, in usual position.

Spermathecae are characteristic in shape; generally, they resemble in shape those of *Ph. phaselus* and of *Ph. maculosus*. The indentations on the surface and peripheral zigzags may be sometimes not so distinct as in the original figure. The diverticulum is usually nearly equal in length to the main portion, but sometimes may be either a little longer or shorter.

Remarks:

The original specimens were poorly preserved and already macerated to some degree, and some characters seem to have been incorrectly described.

Position of male pore on papilla shows slight variation in *Ph. serrata*, i. e. being near the centre or on the lateral part; presence of the annular testis sacs in *Ph. kamitai* was made clear by the present fresh materials.

From these, *Ph. serrata* must be placed under the synonymy of *Ph. kamitai*. Lymph glands are always found behind caecal segment caudalwards, and in *Ph. serrata* inconspicuous ones were rarely also found in front of this region. Such a variation may be of little significance.

Pheretima sempoensis, n. sp.

Sempo, Kôgen-dô, two clitellate and three aclitellate and juvenile specimens, August, '35.

Description:

External characteristics:

Colour in formalin, pale grey dorsally and also ventrally, clitellum fleshy brown.

Shape of the body is very characteristic in all, especially in both clitellate specimens as in Fig. 12, a; it may be one of the characters in the present species. Preclitellar segments as a whole somewhat appear as an elongated ovoid with the largest diameter in VIII; clitellum is markedly swollen suddenly; male segment and its adjacent region is also thick and slightly thicker than the clitellum; posteriorly to the male region it becomes gradually thinner in diameter to about the posterior tenth segment; in this latter region it is suddenly swollen again but thinner than the male segment; the approximate diameter in several segments as follows: 3.2 mm/III, 5.3 mm/VIII, 3.4 mm/XIII, 5.1 mm/XV, 5.6 mm/XVIII, 4.2 mm/XXV, 3.4 mm/LX, 3.0 mm/LXXX, 2.8 mm/LXXXIX, 3.4 mm/the posterior fifth segment.

Length 94 and 98 mm, greatest diameter 5.5 and 5.6 mm, number of segments 102 and 99.

Prostomium, epilobous ca. 2/3.

First dorsal pore in 12/13; but, in both specimens was found an indistinct non-functional pore-like marking in 11/12 respectively.

Setae very small or partly rather delicate; the ventrolateral setae on the spermathecal segments are delicate, so it is difficult to count their number; in general, ventral setae slightly larger and more closely set than the dorsal. Midventral break is usually not found, but when mid-dorsal break is present it is but slight. Setal number as follows: 36, 34/III, 59, 55/VI, 56, 61/VII, 60, 52/IX, 62, 50/XX, 62, 58/XXX, spermathecal setae 29, 32/V, 32, 32/VI, 32, 34/VII, 32, 35/VIII, male pore setae 12 and 15.

Clitellum entire, in XIV–XVI, without setae.
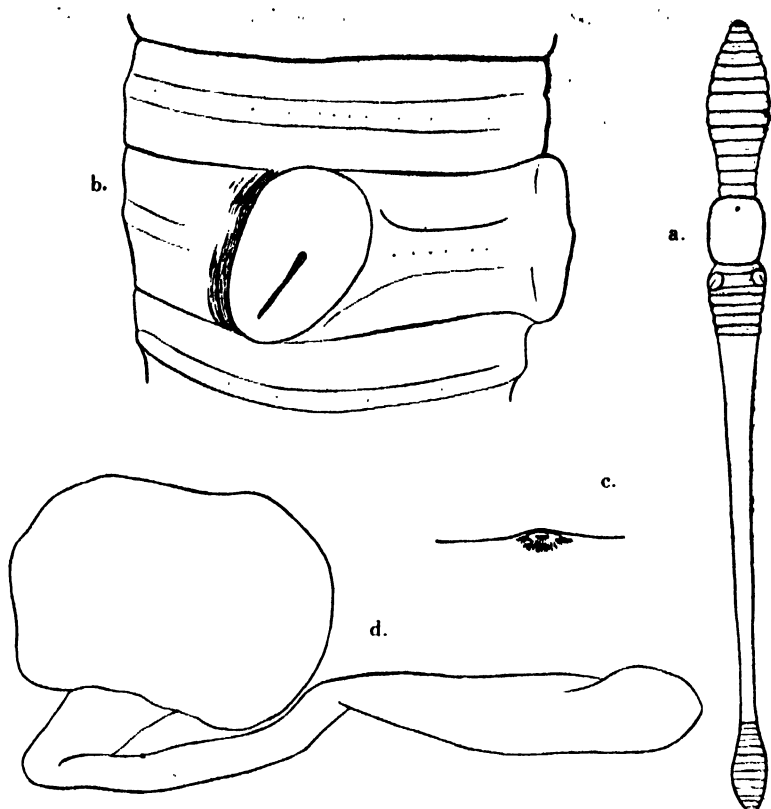
Fig. 12. *Pheretima sempoensis,* n. sp. a, general appearance of body, free hand.
b, ventrolateral view of male pore region, ×ca. 12.6. c, spermathecal pore, ×ca. 22.3.
d, spermatheca, ×ca. 28.

Aspect of male segment somewhat resembles that of *Ph. bitheca*
KOBAYASHI. Male pores ventrally on the setal line of XVIII, about 1/3
of the circumference apart; each pore on a large disc which is oval,
moderately protuberant, nearly flat on surface, and diagonally placed with
the anterior end nearer to the midventral line and fully occupying the
whole segment and more slightly pushing 18/19 posteriorwards. On the
surface of disc a rather distinct groove runs diagonally along its long axis
from the centre where the male opening is situated, to near the posterior
end (Fig. 12, b).

Female pore single, midventrally on XIV.

Spermathecal pores, three pairs in 5/6–7/8; each pair ventrally a
little more than 1/2 of the circumference apart. Strictly saying pores

are situated anteriorly on VI–VIII, very close to the intersegmental furrow, showing a relatively large slit on a minute tip (Fig. 12, c).

No more genital markings found elsewhere.

Internal anatomy:

Septa, in general, moderately thickened; 5/6, 6/7 much, 7/8, 10/11, 11/12 moderately, 12/13 slightly thickened, 8/9 and 9/10 absent.

Gizzard small, barrel-shaped; intestine begins to swell in XV. Position of the intestinal caeca is the most distinct characteristic for the present species; they originate in XXIV, simple, finger-shaped, extending anteriorly into XVII or XVIII; each with rather distinct septal constrictions, and ventrally but proximally with a few serriformed outgrowths.

Hearts in IX asymmetrical, those in X–XIII gradually becoming larger in calibre, of which those in X are rather vestigeal in size and whitish in appearance. Lymph glands inconspicuous but found behind the middle portion of the body caudalwards. Dorsal vessel in the genital region much enlarged, while that of the remaining portion is very small.

Testis sacs in X and XI, both U-shape, remaining as a short cut middorsally, posterior U a little larger than the anterior. Seminal vesicles, two pairs in XI and XII; both pairs very small or rather may be said to be vestigeal in size, or resemble the pseudovesicles in shape and in size, which are usually found in many species of the genus, and are only a little larger than the testes. Vesicles as well as the hearts are not enclosed within the testis sacs. Pseudovesicles not found.

Prostate glands small, occupying about two segments, firmly supported by several fibres which are derived from both ventral and dorsal parietal walls; glandular portion not deeply incised. Duct relatively thick and long to the small size of the glandular portion, with nearly equal thickness throughout its length, looped in an S-shape. Parietes corresponding to the male disc slightly thickened, but no especial structure found there.

Ovaries fairly large, in usual position.

Spermathecae small; ampulla spherical and weakly wrinkled on surface, duct rather short but thick and distinctly marked off from the former; diverticulum always much longer than the main portion, its ectal half slender but thick-walled, the ental half thin-walled, tubular seminal chamber which is a little thicker than the ectal (Fig. 12, d).

Remarks:

The present species is quite distinct from the hexathecal members of the genus in the characteristic position of the intestinal caeca.

Pheretima hupeiensis (MICHAELSEN)

1933 *Pheretima hupeiensis*, CHEN, Contr. Biol. Lab. Sci. Soc. China, Vol. IX, Zool.
     Ser., No. 6, pp. 251–255.
1935 *Pheretima hupeiensis*, GATES, Smithson. Misc. Coll., Vol. 93, No. 3, pp.
1935 *Pheretima hupeiensis*, CHEN, Contr. Biol. Lab. Sci. Soc. China, Vol. XI, Zool.
     Ser., No. 4, p. 121.

Localities :
Kankyô-hoku-dô :   Jôshin.
Kankyô-nan-dô :   Hokusei, Kankô, Shinkôzan, Genzan, Tokugen near Genzan.
    Heian-hoku-dô :   Shingishû, Teishû, Myôkôsan.
    Heian-nan-dô :   Shin-anshû, Chôrin, Heijô, Chinnampo.
    Kôkai-dô :   Shari-in, Ginsen, Kaishû.
    Kôgen-dô :   Kinka, Kôjô, Chûmonshin, Genshû, Bunmaku, Shunsen.
    Keiki-dô :   Keijô, Kaijô, Ryûjin, Suigen, Heiten, Rensen, Monsan, Shin-i, Heitaku, Gumpojô, Chikusan.
    Chûsei-nan-dô :   Gasan, Reizan, Zuisan, Taiden, Chôchi-in.
    Chûsei-hoku-dô :   Seishû, Kwaisan.
    Zenra-hoku-dô :   Kinsan, Kintei, Nangen, Riri, Zenshû.
    Zenra-nan-dô :   Mokpo, Junten, Chintô, Saishû (Quelpart Island).
    Keishô-hoku-dô :   Kinsen, Bunkei, Taikyû, Antô, Hokô.
    Keishô-nan-dô :   Fusan, Kan-an, Kyôsen.
Recently ('36) I reported its distribution in the east-northern part of the Korean Peninsula. As above listed, the present species is widely distributed in Korea, except in the far northern highlands. The present paper also includes its occurrence in Quelpart Island, adding to my preliminary survey of its earthworm-fauna ('37).

It has been reported from Central China, and from Central Japan (and also from Philadelphia, America). But, possibly this peregrine form may be widely distributed in east-northern Asia, as already hinted by CHEN.

In most characters, the Korean form agrees with the Chinese one which was described by CHEN. Length 61–150 mm, greatest diameter 3.5–5 mm, number of segments 97–132. First dorsal pore in 11/12, usually indistinct and sometimes may be non-functional, that in 12/13 always distinct. Setal number between spermathecal pores 10–16/VI, 10–16/VII, 12–18/VIII, male pore setae 10–16, clitellar setae 8–10/XIV, 7–10/XV, 10–16/XVI.

Testis sacs in X and XI, and more, membranous sacs in XII were observed as CHEN's figures show, but his " membranous sacs " in X and

XI must be the testis sacs themselves, and those in XII must be said to be " membranous sacs ". GATES remarks, commenting upon CHEN's description, " He places the seminal vesicles of. . . . , though there is no testis sac in XII ". What is termed to be " testis sacs "? It seems to me to be clear from CHEN's figures that there are no testis sacs in XII, since no testes are found within the membranous sacs there placed.

Pheretima aggera KOBAYASHI

1934 *Pheretima aggera*, KOBAYASHI, Jour. Chosen Nat. Hist. Soc., No. 19, pp. 7–10, pl. figs. 7–13.

Localities :
Kankyô-nan-dô : Eikô, Kôgen.
Heian-nan-dô : Heijô, Chinnampo.
Kôkai-dô : Shari-in, Shinkei, Sainei.
Keiki-dô : Keijô, Sakunei, Rensen, Kôka, Fûhei, Heiten.
Chûsei-nan-dô : Zuisan, Renzan, Reisan.
Keishô-hoku-dô : Taikyû, Shôshû.
Keishô-nan-dô : Fusan, Kan-an.
Description (from fifty specimens from the localities above listed) :
External characteristics :

Length 202–375 mm, greatest diameter 6–9 mm, number of segments 140–189 and usually in the range of 150–160. The body is rather slender, but is much more longer than the other species found in Korea, so the present species is easily distinguishable from the others. Colour in living condition, dark brown dorsally and concentrated on the preclitellar seg-
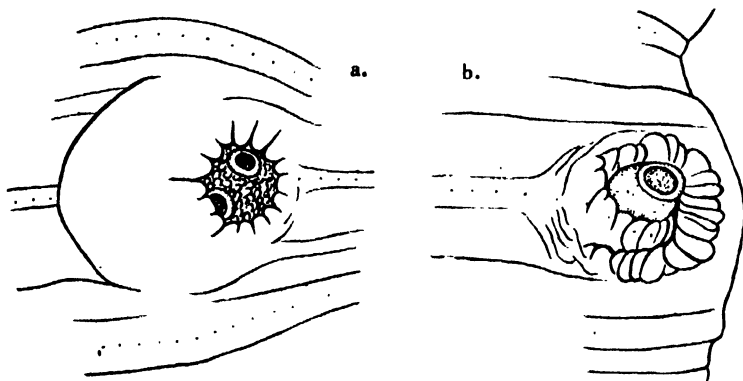
Fig. 13. *Pheretima aggera*, KOBAYASHI. a & b, male pore region. ×ca. 18.9 a, usual form, and b, special case collected from Sainei.

ments, dark grey ventrally, clitellum reddish chocolate. First functional dorsal pore usually in 12/13, seldom in 13/14, sometimes a slight non-functional pore-like marking may be found in 11/12.

Clitellum entire, in XIV–XVI, without setae.

Setae moderate in size; setal number as follows: 34–47/IV, 52–67/XII, spermathecal setae 15–22/VI, 16–23/VII, 16–24/VIII, male pore setae 16–23. 3–4 of the male pore setae are always found on male discs, and may be invisible when the latter are withdrawn into the coelom.

The appearance of the male disc (Fig. 13, a) is usually similar to the original figures; but, only one exception was found in the Sainei-specimens collected in 1936 (=? *Ph. tschiliensis*): the genital papillae on discs are of a single respectively, and this is much larger than the usual ones (Fig. 13, b).

The preclitellar genital papillae are not found infrequently, and show considerable variation. Some instances are given below:

Locality	Position	VII		VIII		IX	
		R	L	R	L	R	L
Zuisan	presetal	0	0	0	0	0	0
	postsetal	0	0	0	0	0	0
Renzan	pre.	0	0	0	0	0	0
	post.	0	0	0	0	0	0
Reisan	pre.	0	0	3	3	3	3
	post.	1	2	6	3	0	0
Fusan	pre.	1	1	2	1	3	3
	post.	0	0	1	0	0	0
Shôshû 1	pre.	1	1	1	1	3	3
	post.	0	0	0	0	0	0
Shôshû 2	pre.	1	1	3	3	3	3
	post.	1	1	1	1	0	0
Sainei	pre.	0	0	0	0	0	0
	post.	0	0	0	0	0	0

Spermathecal pores, three pairs in 6/7–8/9; each just anteriorly with a small spermathecal papilla which is slightly smaller than the genital papillae, and may be deeply sunken into the intersegmental furrow. In the original description I described by mistake the situation of the spermathecal pores as posteriorly on VI–VIII; here, I correct it to be intersegmental.

Internal anatomy:

Septa, in general, considerably thickened; 8/9 ventrally present but thin, 9/10 absent. Hearts small, especially those in X much small in

calibre. Lymph glands moderate in size, found behind caecal segment caudalwards. Testis sacs large, each pair forms a single ventral sac. Seminal vesicles moderate in size, each with a distinct dorsal lobe; when heavily infested by Gregarin cysts they are always voluminous and much larger than the usual size. Pseudovesicles considerably large. Prostates are similar in appearance to each other in all cases examined, and to the original description; but the white spots on the gland which are sometimes found must be Gregarin cysts. Accessory glands corresponding to the genital papillae placed on male discs do not project into the coelom, but are found in the copulatory chamber. Diverticulum of the spermatheca may be said to be moniliform, and is usually slightly longer than the main portion if stretched. Very close to, and just anteriorly to, each spermathecal duct is usually found a small accessory gland corresponding to the spermathecal papilla. Accessory glands which correspond to the genital papillae if present on spermathecal region, are always found internally.

In most specimens were found Gregarin cysts; sometimes heavily infested, and on various parts of the body, mainly in front of the prostatic region. In one heavily infested Heijô-specimen were found abnormal anterior male organs: left half of the testis sacs in X becomes enormously large, nearly equal to the normal size of the seminal vesicles, massive and containing much sexual products; right one of the seminal vesicles in XII becomes vestigial in size, being only a little larger than the pseudovesicles; testes and funnels are situated in usual position, but those of the left side in X much larger than the rest, about 1.8 mm in diameter, while the others about 1.1 mm.

Remarks :

Ph. aggera is very close in many respects to *Ph. tschiliensis* and *Ph. kiangsuensis.* I have thought, as similarly in the case of CHEN's remarks in the latter species, that the present species may be also synonymous with the former. But, I intend to retain the name of the present species until a type of the former species is obtained for examination, since some differences are found between them and this species.

Pheretima quelparta KOBAYASHI

1937 *Pheretima quelparta,* KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 3, pp. 347–350, Fig. 5.

Keishû, Keishô-hoku-dô, four clitellate and six aclitellate specimens, August, '35.

Description:

External characteristics:

Colour dorsally green and concentrated middorsally, lighter ventrally, clitellum russet or light chocolate.

Length 132–160 mm, greatest diameter 6.5 mm, number of segments 107–111. Prostomium, epilobous ca. 2/3; narrow and long. First dorsal pore in 12/13; in one specimen is found a non-functional pore-like marking in 11/12. Clitellum entire, in XIV–XVI, without setae; but in two specimens setal pits were found.

Setae beginning on II, very small or rather delicate; those on posterior end may be slightly enlarged. Both mid-dorsal and -ventral breaks very slight if present. Approximate setal number as follows: 30–36/III, 43–48/IX, 50–58/XX, spermathecal setae 15–17/VII, 16–18/VIII, male pore setae 15–18.

Secondary male pores are formed by large epidermal invagination. Each represented as a large longitudinal slit with a few ridges, especially so on medial side; the region around this opening is gently elevated, forming a cushion-like elevation. Pores are about 3/11 of the circumference ventrally apart (Fig. 14, a).

Female pore, single, midventrally on XIV.

Spermathecal pores, three pairs in 6/7–8/9, ventrally about 1/3 of the circumference apart. Each represented as a large transverse slit in the intersegmental furrow, transversely about three setal wide; within
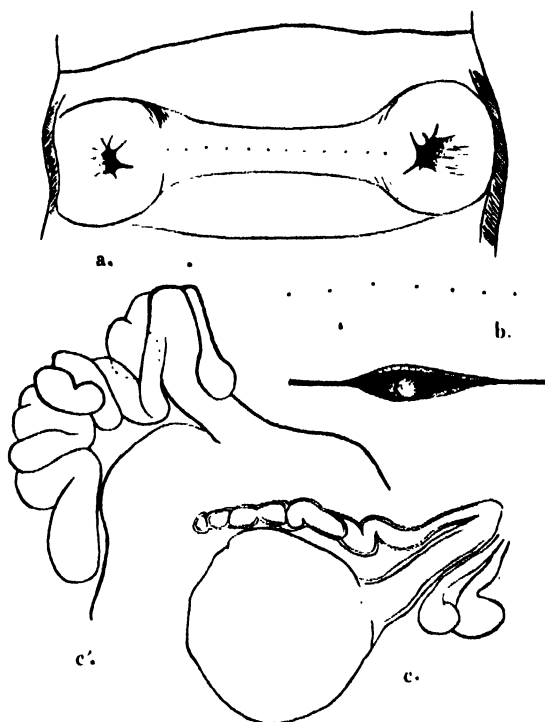
Fig. 14. *Pheretima quelparta* KOBAYASHI. a, ventral view of male pore region. ×ca. 12.6　b, ventrolateral view of spermathecal region. ×ca. 24　c & c', spermathecae with accessory glands corresponding to spermathecal papillae. ×ca. 12.6

this slit is found a saccular body, which is moderate in size and appears only to be a usual spermathecal papilla. When the slit is closed, they are not seen externally (Fig. 14, b).

No more genital markings found elsewhere.

Internal anatomy :

Septa, in general, much thickened; 5/6 and 6/7 very much, 7/8, 10/11–12/13 much thickened, 8/9 ventrally traceable, 9/10 absent.

Gizzard large, globular or bell-shaped; intestine beginning to swell in XV. Caeca simple, in XXVII, extending anteriorly into about XXIII or XXIV, ventrally with several serriformed outgrowths, and with distinct septal constrictions.

Hearts in IX asymmetrically developed, those in X–XIII as usual. Lymph glands large, much lobulated, found behind caecal segment caudalwards. On intestine anteriorly to the caecal segment, branched contractile diverticula of dorsal vessel may be found in two pairs in each segment.

Testis sacs, two pairs in X and XI, each oval or circular in shape; anterior pair forming a low V-shape and posterior pair a rather transverse sac; they are anteroposteriorly in contact with each other. Seminal vesicles, two pairs in XI and XII, fairly large and massive, fully occupying the respective segment; each with a large dorsal lobe. Pseudovesicles not found.

Prostate gland moderate in size, in XVII–XIX. Duct large and long, about 5–7 mm if stretched, looped in most cases in a hair-pin-shape, its ental half rather slender, but the ectal half very much thicker increasing its thickness gradually towards the ectal end. The ectal end enters into the round cushion-like copulatory chamber which is about 2 mm in diameter, and within which no accessory glands are found (externally no genital papillae found).

Ovaries usual, in size and position.

Spermathecae large, in VII–IX; ampulla ovoidal or round; duct rather thin relatively to the size of ampulla, distinctly marked off from, and slightly shorter than, the latter. Diverticulum usually twisted or coiled in its most part, always longer than the main portion if stretched, seminal chamber very small or sometimes not specially dilated. Just close to the spermathecal duct is found one or two stalked small accessory glands corresponding to a saccular body (Fig. 14, c & c').

Remarks :

The present species resembles *Ph. tschiliensis* and *Ph. aggera* in male pores of epidermal invagination, and shape of spermathecae. But, it differs

from both in (1) colouration of the body, (2) spermathecal pore aspects. Its greenish colouration is very characteristic, and is deeper in green than that of the original specimens. The present specimens are identical with *Ph. quelparta*, though slight differences are found.

Pheretima diffringens (BAIRD)

1934 *Pheretima mirabilis*, GATES, Rec. Ind. Mus., Vol. XXXVI, Pt. III, pp. 260–263.

1935 *Pheretima mirabilis*, GATES, Smithson. Misc. Coll., Vol. 93, No. 3, p. 12.

1935 *Pheretima heterochaeta*, CHEN, Bull. Fan Mem. Inst. Biol., Zool., Vol. VI, No. 2, pp. 34–36.

1936 *Pheretima diffringens*, GATES, Rec. Ind. Mus., Vol. XXXVIII, Pt. IV, pp. 412-413.

1936 *Pheretima heterochaeta*, CHEN, Contr. Biol. Lab. Sci. Soc. China, Zool., Vol. XI, No. 8, pp. 270–271.

1937 *Pheretima nipponica*, OHFUCHI, Saitô Hô-on Kai Mus., Res. Bull., No. 12, pp. 108–132, text-figs. 29-36.

1937 *Pheretima diffringens*, GATES, Rec. Ind. Mus., Vol. XXXIX, Pt. II, pp. 198-201.

Localities :

Zenra-nan-dô : Junten, Mokpo, Kwantô.

Zenra-hoku-dô : Zenshû, Riri, Kintei.

Keishô-nan-dô : Sansei, Fusan*, Tôrai*, Urusan*.

Keishô-hoku-dô : Antô*.

Chûsei-nan-dô: Gasan.

Keiki-dô : Keijô.

As CHEN's saying, the synonymy of this world-wide distributed species is intricate. GATES ('34 and '35) placed this as a synonym of *Ph. mirabilis* (BOURNE) 1887, and subsequently ('36) changed the latter into *Ph. diffringens* (BAIRD) 1869. Recently CHEN reported this species from various places in China under the name of *Ph. heterochaeta*, and he ('35) said " I agree with him (GATES), if a more careful survey of the type (*mirabilis*) locality reveals no other form which may cause confusion. But in taking papillae as a criterion, confusion may sometimes ensue " and noted again ('36), " However, the notes from his observation of the type (*diffringens*) are not available at present ". Here, I place this species under BAIRD's name, *Ph. diffringens*. OHFUCHI ('37) reported some variations of the present species under the name of *Ph. nipponica*.

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*These specimens are preserved in the Biological Institute of Tôhoku Imperial University, and their details will be reported by Dr. S. HATAI.

Description :

External characteristics :

Length 80–130 mm, greatest diameter up to 5 mm, number of segments 90–112. Colouration, dorsally reddish brown and setal zone with a faint banded appearance due to the absence of pigment; light brown to pale ventrally; clitellum yellow or reddish orange. Prostomium, epilobous ca. 1/2. First dorsal pore in 11/12, but rarely that may be non-functional, and in one case it was only a slight non-functional pore-like marking. Clitellum entire, in XIV–XVI, without setae; in some apparently mature specimens setal pits were found.

Setal arrangement characteristic as is well-known in this species. Setal breaks on both mid-dorsal and -ventral surfaces slight but found throughout the entire length of the body. Setal number as follows: 24–28/III, 30–35/VI, 39–44/X, 39–49/XX, spermathecal setae 7–10/VI, 7–13/VII, 10–14/VIII, male pore setae 11–16.

Male pore aspects are somewhat variable; usually genital papillae are absent on male pore region, and a small oval or circular porophore found on a large oval or circular, moderate protuberance with a few incompletely circumferential furrows which is placed ventrolaterally on the setal line of XVIII. Sometimes, male porophore and genital papilla are transversely or diagonally coalesced with each other forming a gourd-shape. When genital papillae are present, aspects of male pore region are classified into two groups of CHEN's B and C types ('33).

Spermathecal pores, four pairs in 5/6–8/9, usually inconspicuous. Spermathecal papillae found either anteriorly (usually) or posteriorly to each spermathecal pore, but sometimes they may be lacking. Preclitellar genital papillae found usually on spermathecal segments with 2–4 inter-setal distance between the papilla and spermathecal pore on each side; their occurrence in 24 specimens examined, is given below.

No.	VII R	VII L	VIII R	VIII L	IX R	IX L	No.	VII R	VII L	VIII R	VIII L	IX R	IX L
1	0	0	0	0	0	0	13	0	0	1	1	1	0
2	0	0	0	0	0	0	14	0	0	1	1	1	0
3	0	0	1	1	0	0	15	0	0	1	1	1	1
4	0	1	1	1	0	0	16	0	0	1	1	1	1
5	1	1	1	1	0	0	17	0	0	1	1	1	1
6	1	1	1	1	0	0	18	0	0	1	1	1	1
7	1	1	1	1	0	0	19	0	0	1	1	1	1
8	1	1	1	1	0	0	20	0	0	1	1	1	1
9	1	1	1	1	0	0	21	0	0	1	1	1	1
10	0	0	0	0	0	1	22	0	0	1	1	1	1
11	0	0	0	0	1	1	23	0	0	1	1	1	1
12	0	0	1	1	0	1	24	1	1	1	1	1	1

Internal anatomy :

Septa, in general, moderately thickened; 5/6, 6/7 much, 7/8, 10/11, 11/12 slightly thickened, 8/9 absent or ventrally vestigial, 9/10 absent.

Intestine begins to swell in XVI; caeca simple, conical, in XXVII–XXIV or –XXIII, ventral margin usually rather smooth, but in one case there were found faint incisions, and in most cases with slight septal constrictions.

Hearts in X absent. Lymph glands moderate or sometimes large, found behind 17/18, in one case behind 16/17, caudalwards.

Seminal vesicles, two pairs in XI and XII, usually small, seldom, very small or fairly large, in the latter cases within them are always found a number of Gregarin cysts; each with a distinctly .constricted ovoidal anterodorsal lobe, frequently dorsal lobe is subdivided into two or three smaller ones. Testis sacs large; both pairs ovoidal in shape; anterior pair usually a low V-shape — connected ventromedially with its opposite with a thin bridge, sometimes a low U-shape with a rather wide thin bridge — such a difference may be caused by the varying degree of the contraction of the worms when they are killed; posterior pair thickly communicating midventrally with its fellow forming a rather transverse sac but with a bilobed anterior margin. Pseudovesicles small, two pairs found in all cases examined on the posterior faces of 12/13 and 13/14.

Prostatic glandular portion absent; in one case is found a small gland which is confined to only XVIII, and in the other case quite vestigial ones are found on both sides. Duct always feeble but weakly musculated, looped in a U- or C-shape or rather straight. Accessory glands found near this region are usually burried in the parietes if present.

Spermathecae are also characteristic in these specimens as is well known in the present species. Diverticulum in most cases examined is longer than the main portion. According to OHFUCHI's study on the Japanese specimens, this is also longer than the main portion. But, in the Chinese specimens studied by CHEN, it is about half as long as the main portion ('31) or it is usually shorter than the latter ('33). Such a difference may be however only a local variation; in fact, in the Sansei-specimens it is clearly shorter than the latter. In some of the Zenshû-specimens, globular sacs of the diverticulum which had been observed in Japanese specimens by BEDDARD were found; their structure and contents within them are quite similar to those of the normal seminal chamber, so these sacs are clearly distinguishable from the bodies in any sort which are formed by parasitism; in fact, in a Mokpo-specimen, the latter case

was observed.

Pheretima carnosa (GOTO et HATAI)

1936 *Pheretima carnosa*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 1, pp. 115–138.

1937 *Pheretima carnosa*, OHFUCHI, Saitô Hô-on Kai Mus., Res. Bull., No. 12, pp. 56–62.

Localities:

Shunsen, Kôgen-dô, one clitellate specimen, May, '33, by J. MURAYAMA.

Seishû, Chûsei-hoku-dô, one clitellate specimen, October, '33; an aclitellate specimen, July, '35; by J. MURAYAMA.

Saishû, Quelpart Island, one clitellate and 3 juvenile specimens, April, '35, by J. MURAYAMA.

Pheretima monstrifera KOBAYASHI

1936 *Pheretima monstrifera*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XI, No. 1, pp. 168–171, fig. 11.

Locality: Heiten, Keiki-do.

Pheretima morii, n. sp.

Kunsan, Zenra-hoku-dô, a single clitellate specimen, August, '34.

Zenshû, Zenra-hoku-dô, a single clitellate specimen, August, '34.

Description:

External characteristics:

Length 115 and 118 mm, greatest diameter 4.5 mm, number of seg-ments 101 and 103. Colour in formalin greyish white on both dorsal and ventral side, clitellum light chocolate. Prostomium, epilobous ca. 1/2. First dorsal pore in 11/12, rather small but functional.

Setae beginning on II, moderate in size; setae of Kunsan-specimen are much retracted; both dorsal and ventral breaks found but slight or very slight. Ventral setae of II–XII, especially of III–IX, enlarged, in these segments $a > b > c > d > e \geqq f \ldots$, and $ab > bc > cd > de \geqq ef \ldots$ On postclitellar segments ventral setae may be very slightly larger than the dorsal. Setal number as follows: 26, 28/III, 32, 33/VI, 39, 41/IX, 52, 49/XX, spermathecal setae 9, 12/V, 10, 13/VI, 11, 13/VII, 14, 14/VIII, male pore setae 13 and 14.

Clitellum entire, in XIV–XVI, without setae; but in Kunsan-specimen

setal pits are found around each segment of the clitellum.

Male pores (Fig. 15, a) situated ventrolaterally on the setal line of XVIII, about 3/8 of the circumference apart. Each pore minute, scarcely visible at the centre of a moderately-sized, oval porophore. In Kunsan-specimen, the porophore is of a somewhat conical swelling. Just anteriorly to, and in line with, the porophore, is found a circular, genital papilla which is a little smaller than the porophore and centrally depressed. The male area containing these two markings is slightly elevated from the general surface, with an indistinct and incomplete circumferential furrow except the medial side, and with a few short and indistinct ridges on its lateral side. Zenshû-specimen lacks the genital papilla on male area.

Female pore single, midventrally on XIV.

Spermathecal pores, four pairs in 5/6–8/9, about 3/8 of the cir-
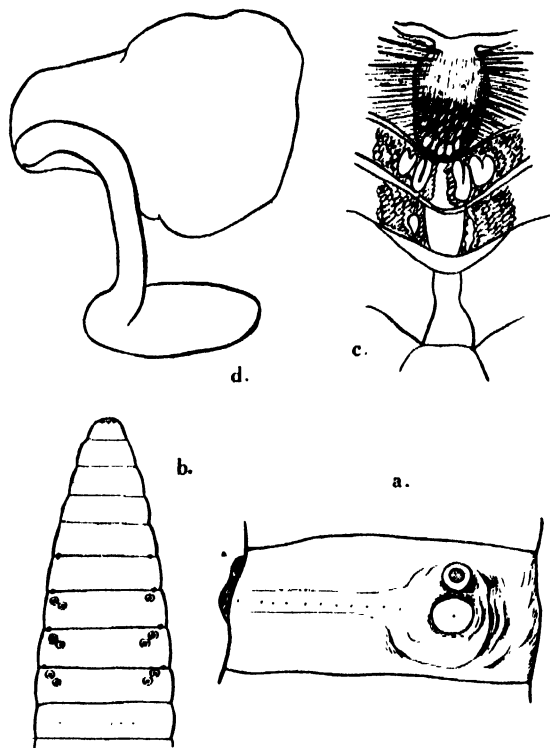
Fig. 15. *Pheretima morii*, n. sp. a, ventrolateral view of XVIII. ×ca. 12.6 b, ventral view of preclitellar segments. free hand. c, pharyngeal region. free hand. d, spermatheca. ×ca. 28

cumference ventrally apart. Each on a tiny tubercle which is sunken into the intersegmental furrow. On each segment of VII–IX, presetally found one or two pairs of genital papillae which are closely similar in size and also in shape to those of the male area. Their arrangement is nearly equal in each case; one pair is closely medial to the spermathecal pore and the other pair closely medial to the former pair to be slightly oblique near to the setal line. There are about 7–8 setae between the inner pair

of the genital papillae. In Kunsan-specimen, on VII one outer pair only, and on each of VIII and IX two pairs; in Zenshu-specimen, on VII one outer pair and one of the inner pair on left side, and on each of VIII and IX two pairs.

Internal anatomy :

Septa, in general, moderately thickened; 5/6, 6/7, 10/11, 11/12 moderately thickened, 7/8 thin, 8/9 ventrally traceable, 9/10 absent.

Pharyngeal glands large; found on pharynx and also in V and VI dorsolaterally on oesophagus. Those in V are larger than those of VI. Each appears to be a large whitish body, as large as about one third of the spermathecal diverticulum, variable in shape, either oval, elongated oval, rounded or miniliform (Fig. 15, c).

Gizzard moderate in size, bell-shaped, being anteriorly narrower. Intestine begins to swell in XV. Intestinal caeca simple, shortly finger-shaped, in XXVII–XXIV, ventrally but proximally only with a few slight indentations.

Hearts IX asymmetrical, those in X–XIII as usual, moderate in calibre. Lymph glands large, fan-shaped, found behind 17/18 caudalwards.

Seminal vesicles, two pairs in XI and XII, oval in shape, small or rather strap-shaped, with a relatively large ovoidal or conical dorsal lobe; dorsal lobe of the anterior pair may sometimes be subdivided into two or three lobes. Testis sacs, two pairs in X and XI, moderate in size; anterior pair a low V-shape with short but rather thick bridge, and posterior pair broadly united; these two of both segments are in contact with each other. Pseudovesicles very small, one pair on posterior face of 12/13.

Ovaries large relatively to the body size.

Prostatic glandular portion entirely absent. Duct was found in all cases examined: very small, short and thin but weakly musculated, and bow-shaped, emerging from the parietes; its ental end is about three times of the vas deferens in thickness and is clearly differentiated from the latter; its ectal end is a trifle thicker than the remaining portion. Close to the ectal end of the duct is found a vestigial accessory gland corresponding to the external genital papilla if present.

Spermathecae (Fig. 15, d) small, in VI-IX; ampulla small, rounded or ovoidal; duct a trifle shorter than the ampulla, very thick and musculated; diverticulum always longer than the main portion, its ental one fourth or one third moderately distended forming an elongated-ovoidal seminal chamber, and the ectal duct portion long and rather thick. Corresponding to the external genital papillae are found large, stalked accessory glands.

Remarks :

The present species stands close to both *Ph. diffringens* and *Ph. divergens* var. *yunnanensis*. (GATES ('34, '35, and '36) classified the latter as synonym of *Ph. diffringens*.) It may be more closely related to the latter, in the presence of genital papillae on VII, VIII, and IX, absence of spermathecal papillae (according to my study in *Ph. carnosa* these papillae are less variable in occurrence than the genital papillae), shape of spermathecal diverticulum, and setal number.

But, it is easily distinguishable in (1) anterior position of the first dorsal pore, (2) two pairs of genital papillae on preclitellar segments, (3) presence of conspicuously large pharyngeal glands, (4) anterior position of intestine and also posterior position of caeca, (5) V-shaped and transverse testis sacs instead of those quite separate from each other, and (6) shape of spermathecae.

It is also distinguishable from the former mainly in (1) detailed aspects of both spermathecal and male pore regions, (2) position of intestine which begins to swell, (3) vascular system, especially presence or absence of hearts in X, (4) number of pseudovesicles, and (5) shape of spermathecae.

The specific name was chosen as an expression of my gratitude to Prof. Dr. TAMEZÔ MORI.

Pheretima murayamai, n. sp.

Junten, Zenra-nan-dô, one clitellate and one aclitellate specimens, March, '35, MURAYAMA.

Description :

External characteristics :

Length 108 mm, greatest diameter 5 mm, number of segments 137 and 139. Colour uniformly yellowish brown on both dorsal and ventral, clitellum blackish brown. Prostomium, epilobous ca. 1/2. First dorsal pore in 12/13. Setae small, beginning on II ; some of every setal ring have fallen and are showing setal pits there. Approximate setal number as follows : 42/IX, 52/XX, spermathecal setae 15/VII, 16/VIII, male pore setae 12.

Clitellum entire, in XIV–XVI, without setae.

Female pore single, midventrally on XIV.

Male pores situated ventrolaterally on the setal line of XVIII, about 3/10 of the circumference apart (Fig. 16, a). Each porophore small, of

a circular papilla; the region around it is slightly elevated and appears to be glandulated and whitish. Immediately posteriorly to, and very slightly medial to, the former elevation, is found a large, circular, gently elevated patch which extends posteriorly to the setal line of XIX, of about 0.8–0.9 mm in diameter. Surface of the patch is somewhat darkened, flattened but appearing slightly glandulated, and is not provided with any special structures. It may be better to say, that the position of the patch is situated pre-setally on XIX, and strongly pushes 18/19 anteriorly into XVIII to be close to the male area.

Spermathecal pores, four pairs in 5/6–8/9, ventrally about 5/11 of the circumference apart; each opening simple, minute but clearly visible in the interseg-mental furrow, due to the presence of whitish margin around it.

Internal anatomy :

Septa, in general, moderately thickened; 5/6, 6/7 much, 10/11–12/13 moderately, 7/8, 13/14 slightly thickened, 8/9 ventrally traceable, 9/10 absent.

Fig. 16. *Pheretima murayamai*, n. sp. a, ventrolateral view of XVIII & XIX. × ca. 12.6 b, caecum. × ca. 12.6 c, prostatic region, free hand. d, spermatheca. × ca. 28

Gizzard small; intestine beginning to swell in XVI. Caeca in XXVII, simple, short and slender, yellowish in colour, extending anteriorly into about a part of XXIV, rather smooth on both margins (Fig. 16, b).

Hearts in IX asymmetrical, those in X and XI enclosed within the

respective annular testis sac, those in XII and XIII as usual. Lymph glands not found.

Testis sacs in X and XI; both annular in shape; anterior ring massive in ventral side but rather thin dorsally; posterior ring much larger than the anterior and containing the seminal vesicles of the segment. Both sacs also containing hearts, both dorsal and ventral vessels, and gut. Seminal vesicles, two pairs in XI and XII, small, nearly equal in shape and in size, each with a small but distinctly constricted ovoidal lobe which is deeply sunken into its anterodorsal surface. Pseudovesicles, one pair, fairly large relatively to the size of the seminal vesicles, ovoidal, darkened in colour, found behind 12/13.

Prostate gland small in XVII–XVIII, darkened in colour, consisting of two main portions, not deeply incised, situated rather dorsally relatively far apart from the position of ectal end of the duct (Fig. 16, c). Duct moderate in length but thin, looped in a c- or l-shape, its ental portion very fine, and gradually increasing its thickness ectalwards, ectal third somewhat thick, musculated and shining. No special structures found inside which correspond to the external genital patch, but very slightly thickened.

Spermathecae in VI–IX, small; ampulla egg-shaped, thin-walled, longer than the duct; duct thin and not sharply marked off from the ampulla; diverticulum large, nearly equal or a little shorter than the main portion, its ental half forming a large spatulated seminal chamber which is somewhat resembling the ampulla in appearance and also in the internal structure, the ectal half slender and not markedly musculated (Fig. 16, d).

Remarks :

The present species is easily distinguishable from the other octothecal members of the genus in the following combination of characters, (1) aspects of male pore region with large genital patches on XIX, (2) annular testis sacs, (3) characteristic appearance of posterior male organs, (4) position of intestine and slenderness of the caeca, (5) shape of spermathecae. In the presence of genital patches on XIX, it resembles *Ph. shimaensis* (GOTO et HATAI); but, differs in many important characters. And, in some characters, it may resemble, however clearly distinct, *Ph. bipapillata* CHEN.

The specific name was chosen as an expression of my gratitude to Dr. JÔZÔ MURAYAMA who kindly sent me the specimens of the present species.

Pheretima sp.

Reisui, Zenra-nan-dô, a single clitellate specimen (its clitellar glandularity not yet complete; posterior part had broken off before it was collected), August, '34.

External characteristics. Small-sized worm, greatest diameter 4.5 mm. Colour has faded away. Prostomium epilobous, ca. 2/3. First dorsal pore in 12/13, distinct and functional. Clitellum ring-shaped, in XIV–XVI; clitellar glandularity not complete, found indistinct intersegmental furrows, but setae and dorsal pores are invisible.

Setae beginning on II, and small; both mid-dorsal and -ventral breaks very slight if present. No marked difference in size between pre- and post-clitellar setae. Ventral setae larger and more closely set than the dorsal. Setal number as follows: 36/III, 60/VI, 63/IX, 65/XX, spermathecal setae 26/V, 26/VI, 27/VII, 28/VIII; male setae 16 if pores are pairly present.

Male pores are found on right side only; two pores ventrolaterally on both segments XVIII and XIX. Two pores are similar in their relative position and appearance. Secondary male pore is represented as a large eye-like aperture which is opened on a large conical protuberance with several ridges. This protuberance as a whole resembles the calyces within which include a bud; and it is transversely 4-5 setal wide at its base, and almost close to the intersegmental furrow with its posterior margin, but anteriorly remaining about one third of the anteroposterior length of the segment. Within the secondary male pore externally is found a relatively large oval-shaped papilla bearing the primary male pore. If the male pores are pairly present, they are about 3/7 of the circumference apart. Just close and anteromedially to each genital marking is found a large dish-like genital patch which is situated presetally but anteriorly slightly pushing the intersegmental furrow and posteriorly cutting the setal line (no setae found between male and genital patch on each side). The surface of the patch is slightly concaved, darkened in colour, and apparently smooth; but, its marginal portion is thickened forming a glandulated and whitish ring (Fig. 17, a).

Female pore single, midventrally on XIV.

Spermathecal pores, three on left side in 5/6–7/8 and two on right side in 6/7 and 7/8, each pair about 3/7 of the circumference apart. Epidermis around each pore is slightly thickened.

No more genital markings found elsewhere.

Internal anatomy :

In general, no septa especially thickened ; 5/6 and 6/7 slightly thickened, 8/9 ventrally present and shoved posteriorly to the posterior side of the gizzard, 9/10 absent.
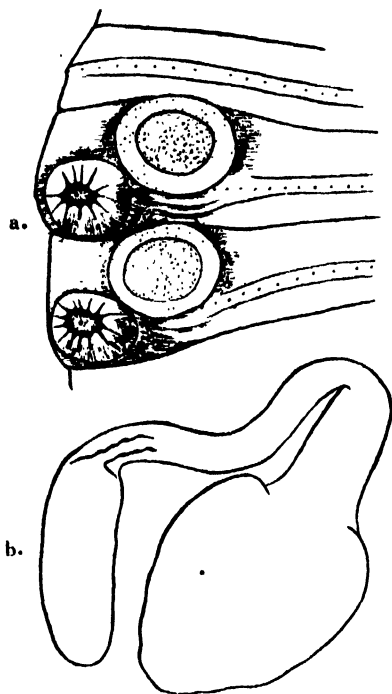
Fig. 17. *Pheretima* sp. a, ventral view of XVII–XIX. × ca. 12.6 b, spermatheca. × ca. 28

Gizzard moderate in size, barrel-shaped. Intestine begins to swell in XV. Intestinal caecum was found on left side only ; in XXVII, consisting of four finger-shaped secondary caeca, of which the dorsalmost is longest, extending anteriorly into XXII, the more ventral ones become gradually shorter.

Hearts in IX asymmetrical, those in X–XIII as usual, small in calibre. Lymph glands found behind caecal segment caudalwards.

Testis sacs very small, two pairs in X and XI ; those in each segment rather widely spaced and appear to be separated from each other ; those of both segments are distinctly separated from each other by 10/11. Testis sac of the right side in X is quite vestigial in size. Seminal vesicles, two pairs in XI and XII ; two vesicles of . the left side small, only situated in the ventrolateral part of the gut, but normal in shape and with a very small dorsal lobe ; those of the right side are quite vestigial in size. Pseudovesicles not found.

Ovaries in XIII, very small ; that of the right side is vestigial in size,

Two prostates are found on right side only corresponding to the external male pores. They are similar in shape, size, and also in structure, except the presence or absence of the connection of sperm-duct with the prostatic duct. Sperm-duct connected only with the ental end of the duct of the first prostate (similar cases are frequently found in *Ph. hilgendorfi*). Each gland consisting of two main lobes, small, occupying about two segments ; parts of the two glands overlapping each other. Duct rather

short but moderate in thickness, looped in a C- or U-shape, both ectal and ental ends thinner than the middle portion. Ectal end enters into a small disc-like glandular thickening and opens through it as a primary pore. Corresponding to the external genital patch, just close and antero-medially to each prostate a large cushion-like glandular mass is moderately protruded into the coelom. Sperm-duct on the left side blindly terminating with a bulbular swelling, as in *Ph. hilgendorfi*, in XVIII.

Spermathecae small, found in VI–VIII on left side and in VII and VIII on right side. Ampulla slightly flattened, heart-shaped, longer than the duct; duct short but relatively thick, moderately distinctly marked off from the ampulla. Diverticulum always a little longer than the main portion, its ental about 2/5 slightly widened forming a seminal chamber (Fig. 17, b).

Remarks :

The present specimen is an abnormal one in considerable degree in many important characters. Although it appears to be clearly distinct from the other species of the genus, it may be advisable to refrain from the erection of a new species name for this abnormal specimen.

Apparently no parasites were found internally. In many important characters were found the multiplication or reduction of the genital organs, such as reduction in number of spermathecae (and caeca), poor development of testis sac, seminal vesicle, and ovary, redundancy on one side for the symmetrical occurrence of prostates.

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STUDIES ON THE COLOR CHANGES OF THE CATFISH, *PARASILURUS ASOTUS* (L.)[1]

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(With thirty-two figures)

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The adaptive pigmentary responses of fish to change in the color or shade of the backgrounds has long been received with a considerable attention by a number of investigators. As to a mechanism concerned in these responses, the recent works of PARKER and his students seem to show that in some fishes there is a hormonal control of the chromatophores, supplementing the nervous control, and that the action of chromatophoral nerves is to stimulate the secretion of such hormonal agents capable to result in the concentration or dispersion of the pigment contained within the chromatophores (PARKER, 1936 a, b). In relation to this aspect the chromatic changes of an American catfish, *Ameiurus nebulosus*, has been recently studied by PARKER (1934 a). The present investigation was stimulated by this aspect and was undertaken as a re-investigation of the subject using the common Japanese catfish, *Parasilurus asotus*, with the view of giving the preliminary ground observations on this fish for further analytical works.

This work was carried on at Asamushi Marine Biological Station during the year of 1936, under the direction of Prof. S. HATAI LL. D., former director of the Station. The writer is greatly indebted to Prof. S. HATAI for his kind supervision and for laboratory facilities provided. Also acknowledgement is due to Dr. Y. WATANABE, Tôhoku Imperial University, who has given many helpful suggestions and criticisms, and has revised the manuscript.

MATERIAL AND METHOD

The fish used as material for the present work were all of second year young ones 10 to 17 cm. in bodylength, and obtained from one locality,

Numasaki, Aomori-ken. These fish only, which were well sensitive to light, were selected with strict attention for all the tests described below.

During the experiments the fish were kept in enameled vessels 25 cm. × 19 cm. by 9 cm. high. Inside surface of the vessels was painted white or black. To prevent their escape, each vessel was surrounded with a net screen 10 cm. high above the edge of the vessel. A Mazuda daylight lamp of 100 watts was employed as a light source, which was suspended at a distance 55.5 cm. above the bottom of the vessel. Under this lamp four vessels were placed together on a concrete sink so as not to give any shade and shadow in the vessels. In order to keep equal oxygen tension, each vessel was supplied with a continuous flow of water of equal quantity, about 800 cubic centimeters per second, from the same tap, of the large laboratory tank. Most experiments described below were performed in a dark room.

For the estimation of phase of body-tint, various stages were recognized, and the following terms were applied after the work of PEARSON (1930): (1) light, (2) light to medium, (3) medium, (4) medium to dark, (5) dark, and (6) black. And for the purpose of graphing the change of body-tint numerical values were assigned as follows: light, 100; light to medium, 80; medium, 60; medium to dark, 40; dark, 20; black, 0. These estimations were made in comparing the tint of fish body with the standard color grades, which were made from a series of sketches of body-tint of fish changing from one extreme to the other.

The examination of the melanophores was made in a definite part of the tail-fin under a compound microscope with both transmitted and reflected light. Type conditions of melanophores were classified into six stages in terminology of HEWER (1926), that is, contracted, slightly stellate, stellate, slightly expanded, expanded and fully expanded. To obtain numerical values which could be expressed graphically as indices of average stage of melanophores in a given area, the following formula was used:

$$\frac{100 \times N_1 + 80 \times N_2 + 60 \times N_3 + 40 \times N_4 + 20 \times N_5 + 0 \times N_6}{N}$$

In this formula, N_1 to N_6 represent the numbers of melanophores which exhibit contracted, slightly stellate, stellate, slightly expanded, expanded, and fully expanded states, respectively. N represents total number of melanophores in the area examined. The coefficients, 100, 80, 60, 40, 20, 0, are arbitrary values assigned to the various stages of melanophores.

RESPONSE TO ILLUMINATED WHITE AND BLACK BACKGROUNDS

General chromatic response and melanophores. In white-walled, illumina-
ted vessel, the fish slowly becomes light brown with a translucent yellowish
or greenish tone, and in black-walled, illuminated vessel, it darkens in
general more quickly to a full black. In either case, the change in body-
tint from one extreme to the other takes place with great rapidity at the
beginning, but, as it progresses, the rate of change decreases and finally
becomes so gradual that it is difficult to determine an exact end-point
(Fig. 1). On the black background the dark tint of fish body continuously
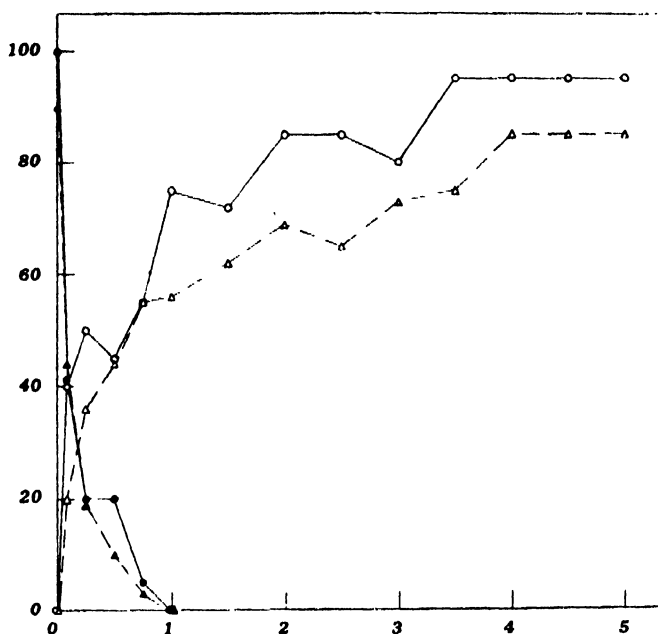
Fig. 1. Graph showing general chromatic change of body: plotted aver-
ages of the data obtained from the experiments on 4 white-adapted fish
and 4 black-adapted fish. Ordinates, indices of body-tint, light state of which
is taken as 100, and black one, as 0; abscissae, time in hours after exchange
of backgrounds. Unbroken lines, changes in white-adapted fish: solid circles,
darkening process; circular outlines, blanching process. Broken lines, changes
in black-adapted fish: solid triangles, darkening process; triangular outlines,
blanching ones.

deepens (solid circles and solid triangles in Fig. 1), but, in the course of
blanching, there are not infrequently found some irregularities in body-
tint at various spots of the body surface (circular outlines and triangular

outlines). The rate of change as a whole differs widely not only in different individuals but also in different conditions, under which the fish were placed before experimentation (pp. 179–183).

As a microscopical observation of the skin shows, the light phase is due to a concentration of the melanophore pigment and the dark one to a dispersion of this coloring matter. As in minnows (HEWER, 1926), the states of dispersion or concentration of melanophore pigment in *Parasilurus* can be classified into six stages ; namely contracted, slightly stellate, stellate,

Figs. 2–7. Large melanophores from caudal fin. Fig. 2, contracted; Fig. 3, slightly stellate; Fig. 4, stellate; Fig. 5, slightly expanded; Fig. 6, expanded; Fig. 7, fully expanded. × ca. 280.

slightly expanded, expanded and fully expanded stage (Figs. 2 to 7 & 8 10). In either dispersion or concentration of melanophores, the initial steps are, as in change of body-tint, always taken with the greatest rapidity, then the rate of change slows down, as it progresses. In other words, on the change from black to light, the process from fully expanded to expanded or slightly expanded stage occurs the most rapidly, and that from slightly stellate to contracted stage, the most slowly ; on the contrary, on the change from light to black, the process from contracted to slightly stellate stage is taken with the greatest rapidity, and that from expanded

to fully expanded stage, with the slowest rate. These characteristics in the course of melanophore change are shown in Figures 11 and 12. In these graphs the average chromatic indices of large melanophores (see below) within the area of a single segment, immediately proximal to that which branches the secondary rays of the fourth or fifth ray in the caudal fin (Fig. 13), are plotted against the time elapsed in hours after the exchange of background.

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From these data, it is also seen that chromatic index of the melanophores as a group decreases not continuously but with some irregular variations near the end of the blanching process. These graphed data suggest that in concentration process, especially in the process from stellate to contracted stage, at least, a certain number of melanophores may pulsate for a while before reaching their ·final stage of concentration, and that such pulsation may not occur in dispersing melanophores. In consequence, it is suggested that the regional variations appeared n the course of blanching of body-tint may be resulted from or regarded as such pulsations of melanophores on the fish body.

9

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Figs. 8 10. Photomicrographs of melanophores in caudal fin. Fig. 8, contracted; Fig. 9, stellate; Fig. 10, fully expanded. × 103. L, large melanophores; S, small melanophores.

The melanophores in this species are of two classes, large and small. In the light phase of the tail-fin, these two classes are easily distinguishable under the microscope. In the contracted stage, melanophores of both
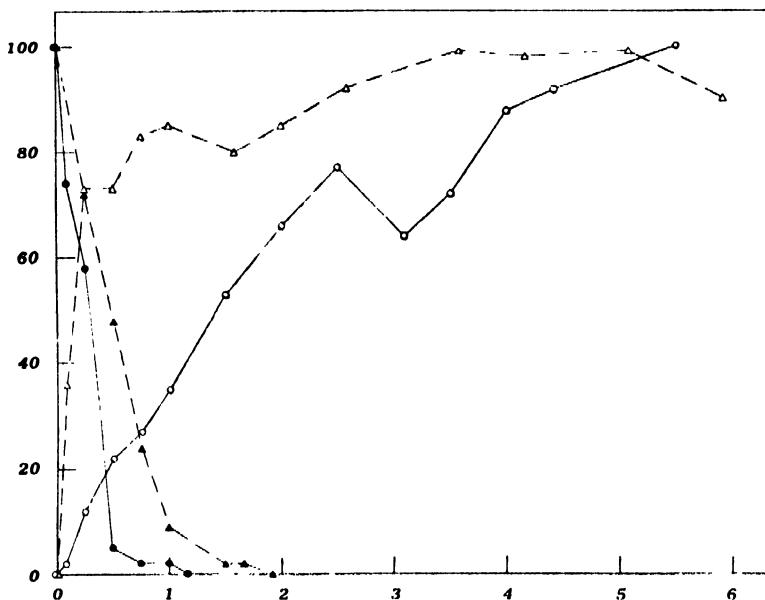
Fig. 11. Changes of large melanophores in the definite single segments of tail-fin rays in two white-adapted fish. Ordinates, indices of average states of melanophores, contracted state of which is taken as 100, and fully expanded state, as 0; abscissae, time in hours after exchange of backgrounds. Solid circles and solid triangles, dispersing changes; circular outlines and triangular outlines, concentrating changes.
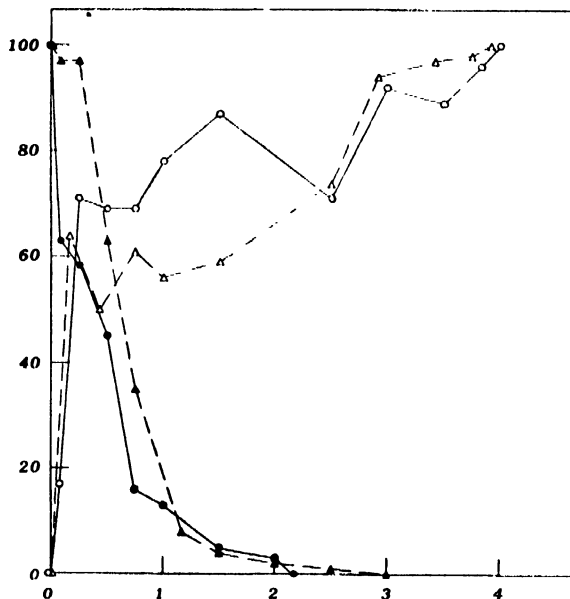
Fig. 12. Changes of large melanophores in the caudal fin of two black-adapted fish. Ordinates, abscissae and symbols, as in Fig. 11.

classes are spherical, but the diameter of the small one is found only one-third that of the large one (Figs. 8 and 14). In the tail-fin, at least, the
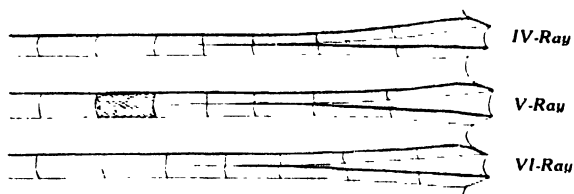
Fig. 13. Diagramatic outlines indicating a segment (shaded in figure) of ray in caudal fin for quantitative observations on melanophore changes. IV, V, VI, ray numbers from dorsal side.

large melanophores rest on the deep surface of dermal layer of the skin, and the small melanophores are exclusively in the epidermal layer, but very few or no melanophores are lodged in the cavities of fin-rays (Fig. 14).
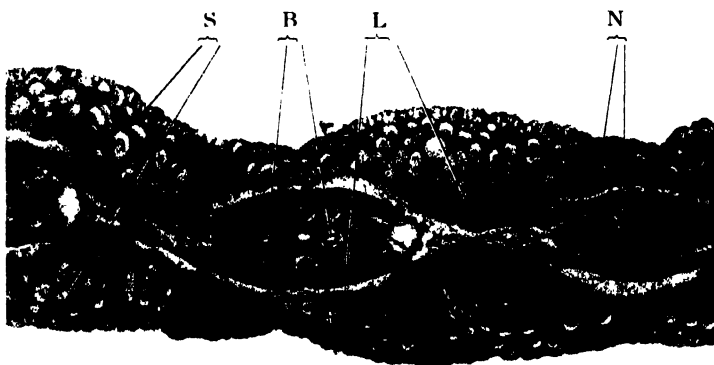
Fig. 14. Cross section of the caudal fin, showing distribution of large and small melanophores. ×88. B, blood vessels; L, Large melanophores; N, radial nerves; S, small melanophores.

Consequently, the distribution mentioned above is not difficult to find out under the microscope with strong light in careful focusing below the surface of the tail-fin. These two classes of melanophores, besides being such histologically different from each other, seem to be so physiologically too. In the tail-fin, the small melanophores response more quickly to the backgrounds (Fig. 15 and Table 1), but are much less affected by the stimulation of transverse section of fin-ray nerves (Fig. 16) than the large melanophores. The small melanophores are small in size; besides, they
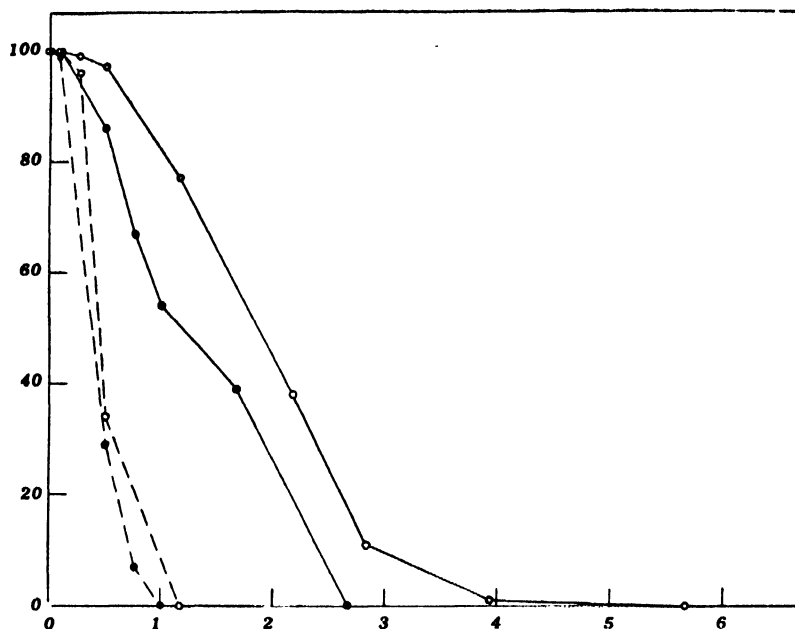
Fig. 15. Dispersing changes on black background in small and large melanophores within the same single segments of the ray: plotted from the data in lot 2 and lot 4 of Table 1. Unbroken lines: changes in large melanophores; broken lines: those in small melanophores. Ordinates and abscissae, as in Figs. 11.

TABLE 1

Time necessary for changes in large and small melanophores within the same single segments of rays

Lot	Large melanophores	Small melanophores
	h. min.	h. min.
1	23 : 55	6 : 54
2	5 : 40	1 : 10
3	6 : 25	3 : 50
4	2 : 40	1 : 00
Averages	9 : 40	3 : 14

distribute by far more sparely as compared with the large melanophores. The change of body-tint is chiefly dependent of the action of large melano-phores, but, needless to say, a part of which may be due to the action
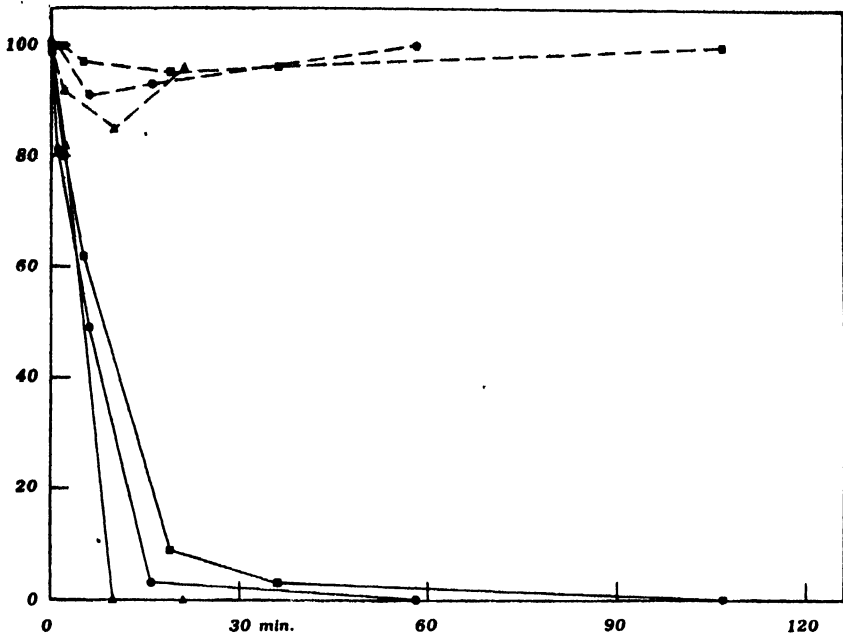
Fig. 16. Effect of nerve cutting on the state of large and small melanophores in the same single segments of tail-fin rays. Unbroken lines: dispersing changes of large melanophores; broken lines: changes of small melanophores. Ordinates, as in Fig. 11; abscissae: time in minutes after section.

of the small ones. In fact, a quick inspection of the tail-fin under the microscope shows clearly the general correspondence in average state of large melanophores with the tint of the part under examination. But the small melanophores appear to react not exactly in the same way as the large melanophores, and often the state of the small ones does not correspond with the general tint there. For these reasons, in the following experiments the large melanophores only were observed.

Effect of repeated change upon the rate of change in body-tint and melanophore state. With a view to establish whether or not the rate of change in general body-tint and in melanophores of the tail-fin is affected by their repeated changes, the fish were placed in white-walled vessels and black-walled vessels alternately. That is to say, light fish were made first to change black in black vessel, then light again in white vessel and so on; and black fish, first to light in white vessel, then black again in black vessel, and so on. In this series of experiments, these fishes were examined, the initial conditions of which were settled as follows:

(1) white-adapted for 10 days in sequence, (2) black-adapted for 8 days in sequence, and (3) white-adapted or black-adapted for 24 hours before experimentation. The results of experiments are graphed in Figures 17 to 19, with average time in hours necessary for change from one extreme to the other in both general body-tint and melanophore state in a definite part of the tail-fin (see Fig. 13) as ordinates against number of times in repetition.

Figure 17 shows the results of experiments on 4 light fish, which were adapted 10 days to the white background. In the graphs, solid circles
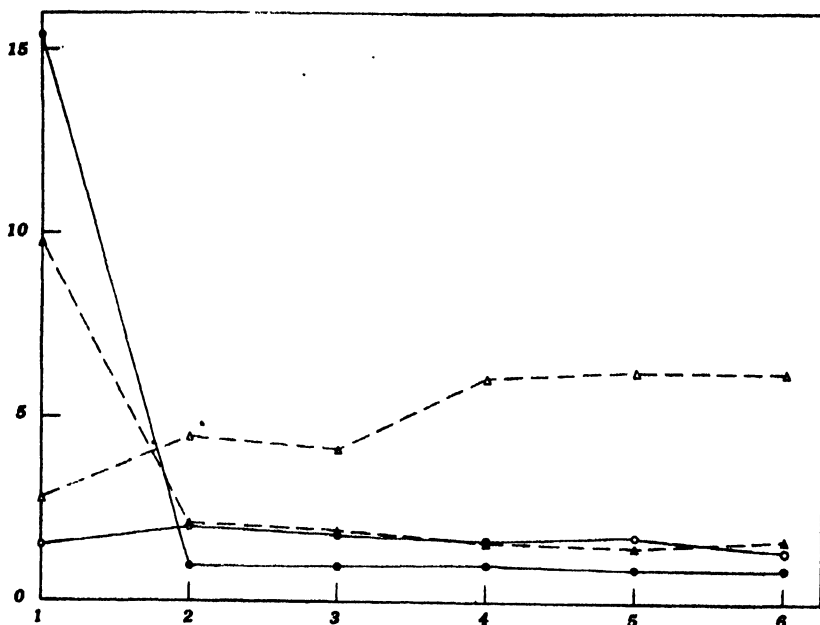
Fig. 17. Time intervals necessary for changes from one extreme to the other in body-tint and in tail-fin melanophores in white-adapted (10 days) fish. Unbroken lines: time intervals for change of body-tint: solid circles: changes from light to black; circular outlines, changes from black to light. Broken lines: the time for changes in melanophores; solid triangles: dispersing changes; triangular outlines: concentrating ones. Ordinates: time in hours for change; abscissae: number of times in repetition.

represent time in hours for change of body-tint from light to black; circular outlines, that from black to light; and solid triangles, time in hours for change of melanophores from contracted to fully expanded state; triangular outlines, that from fully expanded state to contracted one.

In the series of "light to black", the darkening process of body-tint

took some 15 hours in average at the first change, while at subsequent changes, it greatly fastened and showed a certain constant rate (about one hour). On the contrary, in the whole series of "black to light", there was no appreciable variation in the time for blanching from the black tint but nearly constant rate (one hour and half to two hours). In case of the melanophores in the tail-fin, the first dispersing process completed about 10 hours after the fish were placed in the black vessel, and the subsequent dispersing processes, only one hour and half to two hours, but in the concentration series, the first process took nearly two hours, and this time for concentration of melanophores became slightly longer little by little in subsequent concentrating processes.

The results of the experiments on 4 fish, which were kept in black vessels for 8 days before experiments, are shown in Figure 18.
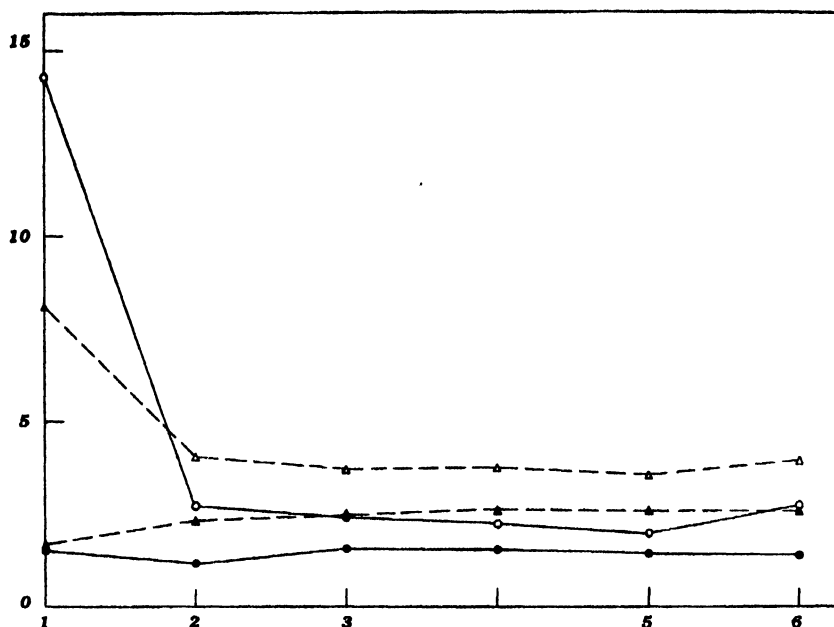
Fig. 18. Time intervals necessary for changes from one extreme to the other in body-tint and in tail-fin melanophores in black-adapted (8 days) fish. Ordinates, abscissae and symbols, as in Fig. 17.

As shown by the graphs, in these fish, the first blanching process of body-tint took about 14 hours in average, but the subsequent blanching processes completed two to three hours after the change of background (circular outlines). The rate of darkening of the fish was nearly constant:

in graph of Figure 20. The graphed data based on averages of the indices of body-tint in five animals as a group.
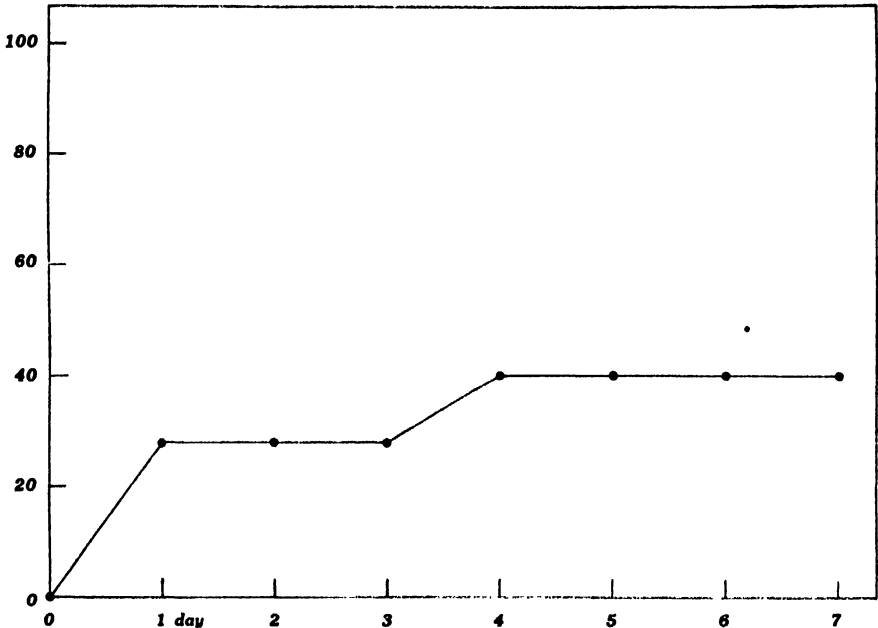
Fig. 20. Graph showing changes of body-tint in darkness; plotted averages of the data on 5 fish. Ordinates, as in Fig. 1; abscissae, time in days after placing fish in darkness.

As shown in Figure 20, it is of much interest that " black " fish does not assume a full black in darkness as might be expected, but it perceptively blanches, reaching some intermediate tone (28 in index) one day after being placed in complete darkness, and it keeps its " medium " tone (40 in index) at least 7 days almost without alteration. The body tint of " light " fish in darkness has not been observed in the present investigation, but according to the findings by ENAMI, normal light fish keeps its light tint more than 15 days in dark environment (ENAMI, unpublished results). Consequently, it is highly probable that in *Parasilurus* a mechanism, with which the light state of body-tint is persisted, is not affected by darkness, but that, which maintains the dark state, is partly unbalanced in darkness.

Optic enucleation and body-tint. In this series the influence of blindness upon the body-tint of fishes on the illuminated white and black back-

grounds, and in darkness were observed.   Five white-adapted light fish and eight black-adapted black fish were employed as material.   Optic enucleation of the fish was made partly under anaesthesia (one per cent solution of ether) and partly without it.

WHITE-ADAPTED FISH.   The removal of eyes causes darkening of fish in illuminated white vessels.   Immediately after operation, body-tint changed to " light to medium "; 35 minutes later one of five animals became " medium ", and four, " medium to dark "; and then on the 5th day all of them assumed " dark ".   This dark tint of blinded fish persisted, at least, 13 days without alteration (Fig. 21).   There was not found any
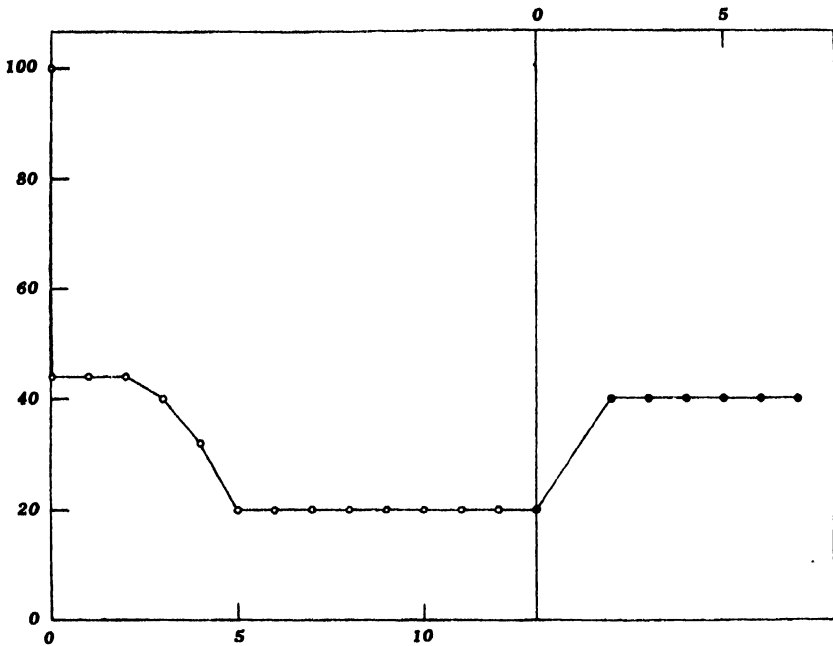
Fig. 21. Effects of optic enucleation and of darkness on the body-tint. Graphed data, based on averages of the data obtained on 5 fish.   Ordinates, as in Fig. 1; abscissae, time in days after removal of eyes (lower scale), and time after putting into darkness (upper scale).

appreciable change in their tone, even when they were placed in the black-walled vessels illuminated by light of the equal intensity.   This final dark tint assumed is not a full black but moderately dark, as shown in most blinded fishes, whereas in an American catfish, *Ameiurus*, is the maximum degree of blackness (PARKER, 1934 a).   However, like *Ameiurus*, these

blinded *Parasilurus* blanch to some extent and show "medium to dark", when they are placed in complete darkness (Fig. 21).

BLACK-ADAPTED FISH. These fish after being blinded remained quite black on either white or black background. When such blinded black fish were placed in dark environment for 10 days in sequence, they became all "medium to dark" tint. However, 55 to 140 minutes after they were put again in illuminated vessels, the tint of these fish returned to a full black; and this maximal blackness persisted without any alteration so long as other conditions were unchanged. From these experiments, we can see that blinded fishes lose their capacity to react to the backgrounds, but they do not lose all of their chromatic response. These facts, in consequence, may suggest the existence of some unknown photosensitive apparatus, besides the ordinary eyes, with reference to the chromatic response.

CHROMATIC RESPONSE TO SECTION OF NERVES

When an integumentary nerve in a light fish is cut, the area thus denervated soon blackens. This is best seen in the tail-fin because it is thin enough to pass light through and it can be easily inspected under the microscope. For the study of the mechanism of melanophore reaction to the denervation by section, the following experiments were undertaken with the tail-fin of the fish. The fish was secured by wet cloth on a wooden board with a tail-fin over a glass plate on the stage of a microscope, and a fin-ray or fin-rays were operated under the microscope at about two-third of the tail length from the edge.

Dark band in the tail-fin. Whenever a transverse cut (ca. 1 mm.) or a small window (0.5 cm by 2.0 mm long) was made in a fin-ray of white-adapted fish, a band of dark color extending distally almost to the edge of tail appeared. As shown by the microscopical examination, the large melanophores in such a band began to disperse in 2 to 120 seconds and fully expanded in 20 to 90 minutes after cutting. In a lot of 8 fish that had been exposed a day or a little more longer to the white background illuminated by a Mazuda lamp before the section was made, the time necessary for which all the melanophores in the band expanded was 38 minutes in average; and in another lot of 7 fish, which had been kept 7 hours in white vessels illuminated by direct sun light, it was 21 minutes in average.

Such a band extends in axial parts of the ray sectioned and across halfway

the interradial space of both sides of the ray, but never across the adjacent regions (Fig. 22). Both in axial and lateral parts the large melanophores were homogeneously dispersed but average rate of their change in the interradial parts is as high as or a little lower than in the axial part. Indeed such a transverse cut of a ray severs both radial nerves and its blood vessels (Fig. 14), but, as a microscopical inspection shows, this cut, excepting within a millimeter or so near it, makes no appreciable disturbance in
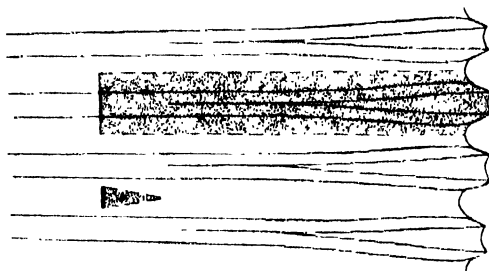
Fig. 22. Diagram of tail-fin rays showing a dark band produced by cutting radial nerves (upper shaded portion), and a short dark triangle made by a cut on interradial membrane (lower shaded portion).

the blood supply to the denervated region. It is naturally so expected because the circulation of blood in the tail-fin consists of ample collateral vessels and their anastomoses. However, the melanophores in immediate neighbourhood of the cut become contracted due to the local anaemia caused by clotting or by the loss of blood through the cut. On a transverse cut made in the interradial membrane, no dark band results, but a short triangle only a millimeter or so in length is produced, the base of which rests on the interradial cut (Fig. 22). This short dark triangle in the interradial space is interesting as contrasted with the dark band formed on axial cut. These results seem to suggest first, that the dispersing fibers to the melanophores are present in the axis of the rays; second, that the innervating area of such dispersing nerves of each ray may be strictly limited within a definite area, which covers an axial part and across halfway the interradial space on both sides, but not overlaps the neighboring regions; and third, that in the interradial space there are no long dispersing fibers, but short side-branches from the axial nerves of each ray.

For the comparative purpose, the same operation was given in five fish of " light to medium " and six fish of " black " in body-tint. In these fishes the dark band was also produced by a cut of fin-ray. When they were placed in illuminated white vessels immediately after cutting, the entire body became pale 15 hours after being exposed to white background with the exception of a denervated band area. But such a band likewise

begins to fade on the white background after having reached its maximum of blackness, and gradually becomes pale. This blanching in the band is much slower than the intact part. According to the results from the examinations of 8 fish, it took 5 to 17 days. This fact may indicate that nerve fibers, which disperse the melanophores, are excited by the stimulation of sectioning and remain active for a considerable number of hours after their severance, even in illuminated white background.
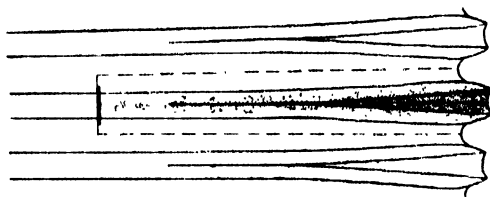
Fig. 23. Diagram of tail-fin rays showing a stage of fading dark band in illuminated white vessel. Fading begins at the lateral margin of the band and proceeds into the axial portion.
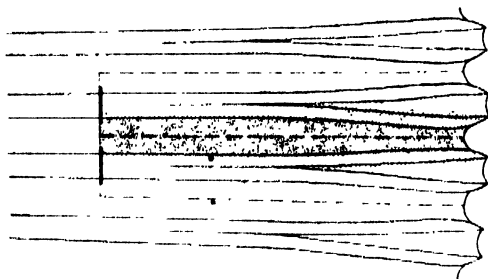
Fig. 24. Diagram of fin rays in which the last part to fade out is the interradial space between two adjoining cut rays.
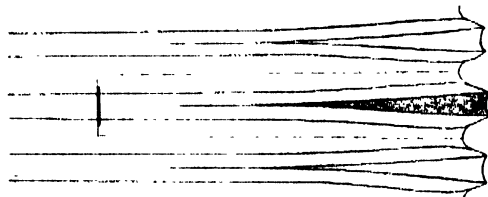
Fig. 25. Diagram of fin rays in which the interradial part between two distal branches of a cut ray is fading out last of all.

Such fading of dark band occurs only when the adjacent part in the tail is in light state, and it appears as if the light neighbouring area spreads into the band from its edges, although general gradual decoloration over a band as a whole is accompanied (Fig. 23). Evidence for such invasion of the blanching process can be seen more clearly in a band, which was made by the section of two adjoining fin-rays. Such a band gradually narrows till the outside of the severed rays has entirely blanched, leaving the interradial space between the two rays still black (Fig. 24). Even in a band on cutting a single ray the last part to disappear is found always to be the interradial area between the two distal branches of the ray sectioned, if it formerly darkened (Fig. 25).

When the light fish with a fully faded band in the tail-fin was placed

in illuminated black vessel, it quickly darkened, excepting the denervated band, which remained pale, for some hours, then it was darkening by far more slowly.

Effect of a second section on the faded band. As the preceding experiments show, a section of radial nerve fibers of a fin-ray results in the darkening of the denervated area distally from the cut, but the dark band thus formed does not extend across the boundaries of the adjacent normal ray-regions. In this series, will be presented the results of the experiments on effect of a second cut made on the same or neighboring rays upon the faded band.

SECOND SECTION ON THE SAME RAY. When a second cut is given at the more proximal level of the same ray to the first cut, the faded band is not again darkened, unless the severed nerves have regenerated at the first-cut surface; but the intervening part between the first and second cut becomes as dark as the dark band produced by the first cut had shown. In most cases, in a completely faded band of this catfish, the second section of the same ray at the distal

Fig. 26. Diagram of fin rays showing the effect of second cut on the completely faded band. A: initial cut; B: second proximal cut; C: second distal cut.

level to the first cut did not show any chromatic change, but in a few cases, in which the initial band faded within 5 or 6 days, such distal band was seen slightly to darken some two hours after the second cut was given (Fig. 26).

SECOND SECTION ON THE NEIGHBORING RAYS. For the examination of effect of a section of neighboring rays upon the faded band, five adjoining rays of a caudal fin in fully blanched fish were used. After the second ray out of the five had been cut, the fish was placed in an illuminated white-walled vessel for two weeks in sequence until its dark caudal band completely faded; then second cuts were given to the first, third and fifth rays at the same time, but the fourth ray remained intact. In the first lot of experiments, the second cuts were made at the same level of the above mentioned rays as the first cut in the second ray (Fig. 27); in the second lot, at more proximal level to the first cut (Fig. 28); and in the third lot, at distal level to the first cut (Fig. 29). The blood in the faded
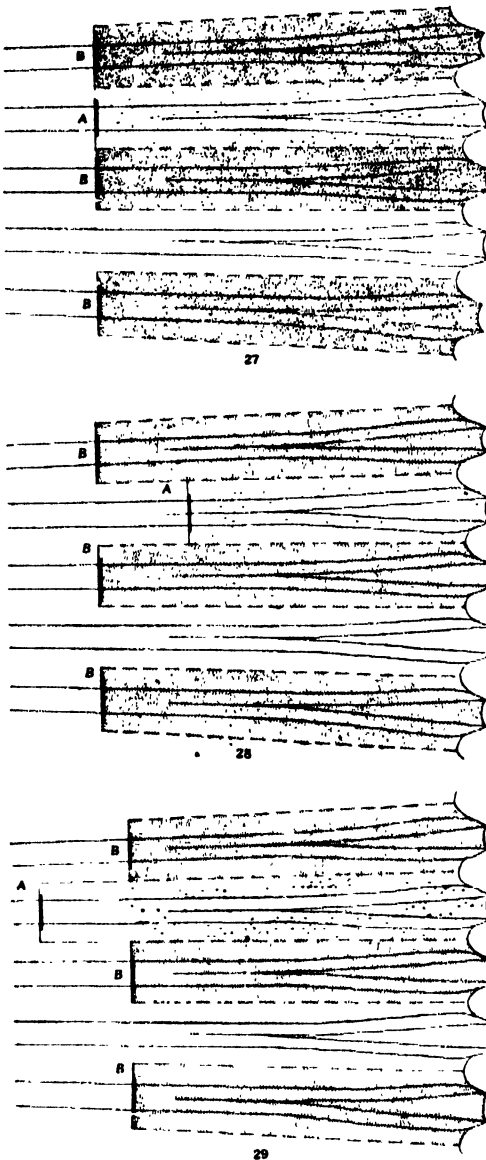
Figs. 27-29. Diagrams of fin rays showing effect of newly excited flanking bands upon a faded band. A: initial cut; B: second cut. Second cut, given at the same level (Fig. 27), or at the more proximal (Fig. 28) or at the more distal (Fig. 29) level to the first cut.

band was found by the microscopical inspection to be circulating almost normlly, even after such second sections were made. The results of the experiments are shown in Figures 27 to 29.

In each lot, as has been expected, three dark bands are produced on the second cutting, and no change in tint of intact fourth ray-region occurs. But the faded band of the second ray perceptibly darkens and persists its dark shade. However, such darkening process in the faded band does not extend proximally beyond the first cut (Figs. 27 and 28). But, when the second cuts of the neighboring rays are made at more distal level to the first cut of the second ray, the part of the faded band distal to the second cut darkens, whereas the faded part proximal to the second cut remains independently pale (Fig. 29). In other words, adjacent newly excited dark bands may bring about dispersion of melanophores in the faded band, but their effect is limited only within the area of the denervated band region distal to the level of

the second cut.

The darkened area of the faded band never becomes so dark, as the flanking bands, but it remains persistently dark as compared with the rest of the caudal fin. The darkening process in the faded band is less rapidly at the axial part than at the lateral parts of the band; in the lateral parts the melanophores begin to disperse almost at the same time as the flanking bands are formed. And in the distal portion of the faded band the axial part darkens much more slowly than the lateral ones. If these fishes are still continuously in white vessel, all the bands there finally fade away. This fading in the flanking bands begins to occur always in these lateral parts which are boardering on the innervating area, and they gradually narrow, leaving the adjacent part to the faded band dark (Fig. 30). These changes in tint are also clearly seen in a faded band with a single flanking band (Figs. 31 and 32).

Fig. 30. Diagram of fin rays showing fading process in two flanking bands and one old band. A: initial cut; B: second cut.
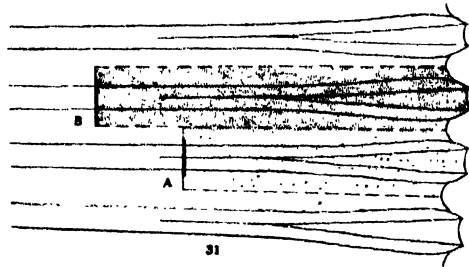
31
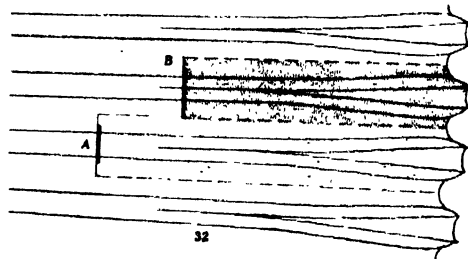
32

Figs. 31-32. Diagrams showing re-darkening in a faded band which is induced by a flanking band. A: initial cut; B: second cut. Second cut, given proximal (Fig. 31) or distal (Fig. 32) to the initial cut.

DISCUSSION

The present studies, conducted with the common Japanese catfish, *Parasilurus asotus*, have dealt with the responses of the

melanophores to the backgrounds and to dark environment in both normal and blinded fishes, and also with their responses to the stimulation by section of melanophore nerves.

The capacity of this species for chromatic adjustments proves to be rather remarkable as of the catfishes, *Ameiurus melas* (PEARSON, 1930), and *Ameiurus nebulosus* (PARKER, 1934 a). *Parasilurus* becomes light in illuminated white vessel, and dark or black in illuminated black vessel. Like *Ameiurus*, these color changes of body are due to a concentration or dispersion of the melanophore pigment in the skin.

As in *Ameiurus* (PARKER, 1934 a), the melanophores in this catfish are of two classes, large and small. The large melanophores distribute in the dermal layer and the small ones, in the epidermal layer of the skin. In contracted state, they are both spherical, but the diameter of the small ones is only one-third of the large ones. According to the observations by PARKER (1934 a), in *Ameiurus* these two classes of melanophores appear to react exactly in the same way. But in this species, besides the histological difference, they are easily distinguished by their reactions. In a caudal fin, at least, the small melanophores respond more quickly to the backgrounds (Fig. 15 and Table 1), but they are far less affected by the stimulation of section of nerve fibers in the fin ray (Fig. 16), than the large melanophores. Moreover, the small melanophores distribute much more sparely as compared with the large melanophores. In fact, the change of body-tint depends almost entirely upon the action of the large melanophores. But concentration and dispersion of the small melanophores occur without bringing about corresponding quantitative changes. From these results, it seems unquestionable that these two classes of melanophores are considerably different from each other, either in mechanism of their movement or in factors controlling it. Consequently, in relation to the further analysis of the melanophore activity in this fish, it is probably not insignificant to point out these differences in physiological characters so distinguishable between the large and small melanophores.

Generally speaking, darkening process of the fish on the black background takes place more quickly than blanching one on the white background. However, the time intervals necessary for change from one extreme to the other are different according to the different conditions, under which the fish were formerly placed. It has been noticed in various fishes by VAN RYNBERK (1906), SUMNER (1911), MAST (1916), PARKER (1934 a) and others that the time intervals for change in body-tint are much abbreviated by repetition, as though the fish acquire their power of chromatic change

more rapidly than before. The data presented in this paper on *Parasilurus* (pp. 179–183) are generally in agreement with the findings by the above mentioned investigators. Such abbreviation in the time interval has been clearly seen for darkening process in the white fish, which were adapted for 10 days to the white background, and for blanching process in the black fish, which were exposed to the black background for 8 days in sequence before experimentation (Figs. 17 and 18).

This remarkable abbreviation, however, does not always take place in this species. As shown in the foregoing Figure 19, in the black fish adapted 24 hours to the black background, the similar abbreviation appears for the blanching change, whereas, in the white fish adapted 24 hours to the white background, no abbreviation for the darkening change occurs: in the latter case, the first darkening process is accomplished in relatively short time, and the subsequent darkening processes are also completed nearly in the same length of time as the first one (Fig. 19). In the other words, the time for the first darkening process in the white-adapted fish increases with an increase in the exposure time to the white background, while in the black-adapted fish the first blanching process seems not to be affected by the length of time of exposure to the black background, for which is always necessary a certain considerable number of hours. And in all cases of both black-adapted and white-adapted fishes the subsequent processes show a nearly uniform condition, in which the change from light to black is attained more quickly than that from black to light. In changes of the melanophore action, the corresponding relations to the repetition are also observed, except in concentration of the melanophores in white fish adapted 10 days to the white background, in which the time interval for the repeated changes of concentration becomes slightly longer, instead of its abbreviation (Fig. 17). This is the only case among all the experiments here attempted, in which the change of general tint and of large melanophores in the tail-fin did not show close parallelism. On this discrepancy no definite reason can be given here, since further comparative data necessary for the account are not at hand.

In these changes, as PARKER (1934 a) has stated on *Ameiurus*, the initial step is always taken with great rapidity, and, after that, the rate of changes slows down (Figs. 1, 11, 12, 15). On the black background, the body-tint increasingly darkens and the dispersion of melanophores continuously takes place, but on the white background the course of the changes in this species is not smooth. As shown in Figure 1, in the course of blanching, the fish often show some back-and-forth changes

of body-tint, that is, at first it becomes light to some extent, later partly darkens, then pale again, and so on ; thus finally it completely blanches. The melanophore group in the tail-fin also shows such variations in index indicating their average states during the course of concentration (Figs. 11, 12). This result of observation seems. to suggest that, at least, a certain number of melanophores of the group may exhibit some pulsatory movement before reaching their final state of concentration. The pulsation of chromatophores in isolated scales of various fishes has been observed by many authors (GILSON 1926 ; SMITH, 1930, 1931 a ; SMITH and SMITH, 1934, 1935 ; SPAETH, 1913, 1916 ; YAMAMOTO, 1933). Referring the observations by these authors to their own results of experiment on the denervated part of the body in *Fundulus*, PARKER and PUMPHREY (1933) have come to conclude that " the melanophore pulsation is an activity that may be carried on by the melanophore itself and that the excitation of this activity results from a direct stimulation of the melanophore and in no sense necessary involves nervous element " (p. 330). If this conclusion hold true in *Parasilurus*, the above mentioned pulsation-like movement of melanophores should be of considerable interest for the study of the the mechanism of concentration of the melanophores in this catfish. According to these authors, in the pulsations of *Fundulus* melanophores, the cell movement in the denervated area reaches from full concentration to about half or three-quarters of full dispersion, but never reaches a full dispersion. This may be also comparable with the phenomena in the blanching course of *Parasilurus*, since, as Figures 1, 11 and 12 show, the back-and-forth changes appear only after the body-tint has attained to some extent lighter or the melanophores have more or less contracted, but no such changes are found in the darker (black to dark) state of body-tint, or in the dispersed (expanded to fully expanded) state of melanophores. At present, needless to say, there are some doubt whether the phenomena under discussion is correspondent to the chromatophore pulsation in isolated scales or in the area of body in which all the nervous elements have been removed by a degenerative operation. However, in view of the observations obtained here, still this peculiar pulsation-like movement of melanophores in the blanching course of the fish may be worth taking into consideration.

From the preceding results from the experiments on effects of optic enucleation and of darkness upon the body-tint of fish, it is obviously seen that the eyes of the catfish are an effective receptor for its chromatic change, as was long maintained by LISTER (1859), and recently advocated

for the catfish, *Ameiurus*, by PARKER (1934 a), PARKER, BROWN and ODIORE (1935), and ABRAMOWITZ (1936).

As has already been noted in the minnow and the carp by VON FRISCH (1911), in the killifish by PARKER and LANCHNER (1922), and in the catfish by PARKER (1934 a), when a normal *Parasilurus* of "black" tint is placed in an environment of complete darkness, it perceptibly blanches and remains its "medium" tint, but never assumes black as might be expected. And when the fish, either light or black in tint, is blinded, the distinction in response to illuminated black and white backgrounds is entirely obliterated, and it becomes dark irrespective of the tint of surroundings. These black blinded fishes if placed in a dark environment become "medium to dark" in tint, but when they are taken back in illuminated vessel, the darkening of body again occurs whether the vessel has white or black wall. These observations on *Parasilurus* are well in accordance with those, conducted with *Fundulus*, by PARKER and LANCHNER (1922). All these interesting results mentioned above make it clear that there must be significant differences in effect on the color changes of these fishes among black background, complete darkness and optic enucleation. For the adaptive responses to the tint of background, the functional eyes are of most importance and they look to be indispensable. However, since the fishes do not lose all of their capacity to undergo melanophore changes even after the removal of eyes, the photosensitiveness in the skin (VAN HEUSEN, 1917; PARKER, 1934 a) or elsewhere in the body surface should never been lost sight of in discussing the color change of fishes. But stimulation initiated by such photosensitiveness does not cause the ordinary chromatic response of body; it may be concerned only with the variations of light intensity.

Following a transverse cut of caudal fin-ray, a dark band is formed, in which the pigment of melanophores is fully dispersed. Such bands, originally employed by WYMAN (1924) for the study of melanophore activity, have been also observed in the present work. As has been mentioned, excepting immediately around the cut, the dark bands thus made are no necessary sense dependent on the disturbances of blood supply. And the dark tint extends from the cut almost to the free edge of the fin, and covers a definite area corresponding to that of distal distribution of the severed nerves, but it never extends across the adjacent intact ray-regions, nor proximally to the cut level (Fig. 22). Consequently, the formation of band is unquestionably under the nervous control. When the fish is kept in a white vessel, this dark band finally fades out, but its

dark tint persists for a considerable number of days after the rest of body has completely blanched. Not infrequently is found that the band does not disappear on the white background until 17 days after severance of the nerves. During such long period of time the degeneration of radial nerves probably may set in along the whole length of the band. In fact, in most cases it was difficult to form a secondary dark band within the area of faded band by a new transverse cut made across the same ray and somewhat distal to the first one (Fig. 26). However, the disappearance of the dark band may not always be associated with degeneration of the band nerves. It is because, in a few cases examined, in which the band faded out 5 or 6 days, a secondary dark band appeared within the area of the old band on the second transverse cut of the ray. Though the material was limited, this result of the experiment seems to clear. In a recent work on *Fundulus* bands, PARKER (1934 b) concludes that the dark band produced by the nerve cutting is not the result of paralysis, as has been maintained by most of the previous investigators, but it is due to the effect of prolonged activity (superactive hypothesis) of severed nerves, which is set up in the cut whereby the nerves are activated. If this is the case in *Parasilurus*, the " superactive state " of the melanophoral nerves in this fish may be said in general to last so extraordinary long time (more than two weeks) as compared with those in *Ameiurus* (PARKER, 1934 a) and *Fundulus* (PARKER, 1934 b), in both of which it may continue at most only a few days.

When the white-adapted fish with a fully faded band is placed in a black vessel, the whole fish darkens rapidly, but the band area darkens a little more slowly than the general surface of the body. In either cases, fading or darkening, was it never found that the tint of the denervating band took place more quickly than the rest of body. In other words, the dark band begins to fade, as SMITH (1931 b) pointed out in *Phoxinus*, only when the intact innervating area maintains its light tint in a white-walled vessel, and the faded band, to darken only after the rest of the caudal fin has changed dark in a black vessel. And, as has been noted by PARKER (1934 a, b), such change of tint in the band always occurs first at the lateral edges of the band area, which are boardering on the intact area, and progresses into the axial part of the region (Figs. 23–25). These results show clearly how dispersion and concentration of melano-phore pigment in the denervated band area may be brought about by adjacent innervating area around it. This fact may be more clearly seen in the white-adapted fish with two new bands made by cutting the fin-rays

adjacent to that of the faded band (Figs. 27 to 30). In such faded band as flanked on each side by newly produced dark bands, the darkening process appears as if the dark tint of the two adjacent bands extends lateraly into the faded band from its edges. When the fish with these three adjoining dark bands is kept in an illuminated white-walled vessel, the fading occurs first in two outer bands and last in the middle one. In the flanking bands, such fading process begins at the lateral edges adjacent to the innervating area, and the last part to fade out is the lateral parts along sides with the initial (middle) band (Fig. 30).

However, darkening of the faded band thus induced is limited within the area distal to the flanking bands. Accordingly, if the second cut on the adjacent rays is given distal to the initial cut of the old (middle) band, the distal part flanked by newly excited dark bands perceptively darkens, whereas the part of the faded band proximal to the flanking bands remains light (Fig. 29). On the contrary, if the second cut is made on the adjacent rays proximal to the initial one, the whole denervated part of the faded band becomes dark, and the innervating area of the middle ray-region between the flanking bands remains independently unchanged (Fig. 28).

In the foregoing pages considerable space has been devoted to the discussion on the phenomena observed in *Parasilurus*, chiefly comparing with the corresponding phenomena in an American catfish, *Ameiurus*, by PARKER (1934 a). So far as the data presented in this paper are concerned, the results of the experiments on the chromatic responses in this fish are pretty well in accord with PARKER's work on *Ameiurus*, and seem to favor his " neurohumoral theory " of chromatophore activity (PARKER, 1932, etc.). However, at present, this aspect of the physiology of chromatophores may scarcely be considered so simple as it seems to be in the above mentioned " neurohumoral theory ". Generally, color change in animals is rather complex. Even in fishes, the color change has been found to be consisted not only of independent responses by the different types of chromatophores, but of different mechanisms which may bring about changes of chromatophores (see, for instance, SUMNER, 1934). Therefore, in spite of the similar results obtained, it may be as yet premature to conclude, from such limited observations as mentioned above, that the comparative chromatic effectors in these two related fishes are under the control of the same mechanism. Although it is indeed of great interest to discuss the foregoing observations from the neurohumoral viewpoint, the writer wishes to reserve any definite conclusions on this fundamental problem until the

further analytical data are sufficiently obtained on this· catfish.

SUMMARY

1.   Upon the Japanese catfish, *Parasilurus asotus* (L.), the effects of background are rather striking.   On the illuminated black background the fish quickly darkens, and on the illuminated white background it more slowly becomes light with yellowish or greenish tone.   Chromatic effectors in this fish are skin melanophores.   The dark phase is due to a dispersion of the melanophore pigment and the light one to a concentration of this coloring matter.

2.   The melanophores in this fish are of two classes, large and small. The large melanophores are in dermal layer of the skin, and the small melanophores in the epidermal layer.   The response to the black background is more quickly and that to the stimulation by cutting the nerve fibers is by far less or almost no sensitive in the small ones than in the large ones.   The change of general tint of body depends almost entirely upon the action of the large melanophores.   The action of the small melanophores takes place without bringing about corresponding quantitative changes.

3.   In the darkening process of body-tint and the dispersion of melanophores on the black background, the changes continuously proceed, while in the blanching process and the concentration of the melanophores, some pulsation-like back-and-forth changes are usually concurred.

4.   Generally speaking, in either fishes, white-adapted or black-adapted, the time interval necessary for the change from one extreme to the other is considerably abbreviated by repetition.   In repeated changes, it shows nearly constant length of time for each (darkening or blanching) change for each fish.

5.   However, the time for the first darkening change in white-adapted fish is different according to different lengths of time for which the fish has been exposed to the white background, and it greatly lengthens if after the long exposure (10 days).   On the contrary, the time period for the first blanching change in the black-adapted fish looks to be considerably long irrespective of the length of such exposure time to the black background.

6.   Similar effects of repetition and of length of exposure time to the same background upon the time periods for the changes have been also observed in the melanophore action in· the tail-fin.

7. In the black-adapted fish, the tint of body, if transferred in the complete darkness, perceptibly blanches, whereas the optic enucleation does not cause any blanching of body-tint both in white-walled vessel or in black-walled vessel. Indeed the optic stimulation from the background through eyes is of most importance, for the adaptive responses, but, besides the eyes, there must be some other photosensitive receptors, which may be effective to the chromatic changes and sensitive only to the change of light intensity of the environment.

8. A short cut, or cutting a small window, across a ray of caudal fin of the white-adapted fish results in the darkening in an elongated denervated area extending from the cut almost to the free edge of the fin. Such dark bands may persist 5 to 17 days after severance of the nerves even if they are exposed to the illuminated white background.

9. The fading of the dark bands and the re-darkening of the faded bands occur with the general changes of body tint as a whole. In either cases, fading or darkening, the change in a band area begins at the lateral parts, which are boardering on the innervating area around it, and it progresses into the axial part.

10. The darkening of the faded band is also induced by newly excited ·flanking bands. The darkening area is strictly limited within the denervated part distal to the flanking band, but the part of the faded band proximal to flanking ones, when there is, independently remains light,

11. The fading of such flanking bands always occurs first at their edges facing to the innervating light area, and extends laterally toward the sides of the initial band, which fades out last of all.

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OCCURRENCE OF *PERIONYX EXCAVATUS* E. PERRIER IN NORTH FORMOSA.

By

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(With one figure)

(Received June 17, 1938)

At my suggestion, Mr. MASAO NAKAYAMA kindly sent me a number of earthworms collected in March, 1938, in Shinchiku, North Formosa. Among them was included a single clitellate specimen of *Perionyx excavatus* E. PERRIER. The chief home of the genus *Perionyx* is the Eastern Himalayas, including the Abor Country and Assam (STEPHENSON '23). *P. excavatus* is a peregrine form which has also been found from the islands and coasts of the Indian Ocean and the Malay Archipelago. The present record is the first from Formosa.

I am greatly indebted to Mr. YAMANAKA for kindly supplying the material.

Perionyx excavatus E. Perrier 1872

1933 *Perionyx excavatus*, GATES, Rec. Ind. Mus., Vol. XXXV, Pt. IV, pp. 549 551.
1936 *Perionyx excavatus*, GATES, Rec. Ind. Mus., Vol. XXXVIII, Pt. IV, p. 466.

External characteristics :

Body slightly compressed dorsoventrally; at first glance, the general appearance resembles that of some Lumbricid species. Length 162 mm, greatest diameter (in clitellar region) 4 mm, number of segments 131. Colour in formalin, dorsally purplish red, preclitellarly deep in purple and with greenish iridescence, middorsally also concentrated in purple along nearly the whole length of the body, ventrally pale or lighter than the dorsal in the preclitellar region; clitellum yellowish grey, dorsally with a very faint purple. The colouration of the dorsal and ventral surfaces is clearly differentiated in the lateral line. Prostomium, epilobous ca. $\frac{1}{2}$. First dorsal pore in 5/6, distinct and functional; pores in 10/11–12/13 are all non-functional but distinct; that in 17/18 functional.

Setae beginning on II, delicate; ventral ones much more closely set than the dorsal, but no marked difference in size found between them.

Middorsal breaks present, zz=ca. 1.5–1.8 zy; midventral breaks are also found in most of the postclitellar segments but are very slight. Setal number as follows: 39/III, 47/V, 53/IX, 51/XII, 52/XX, 48/middle portion of the body, spermathecal setae 4–6.

Clitellum ring-shaped, in XIII–XVII (=5). Circles of setae are present on all segments, and the intersegmental furrows and non-functional pore-like depressions are faintly indicated.

The male apertures are not definitely identified. The male area (Fig. 1, a) is a small and shallow, somewhat transversely rectangular, midventral depression in the setal line of XVIII which does not reach
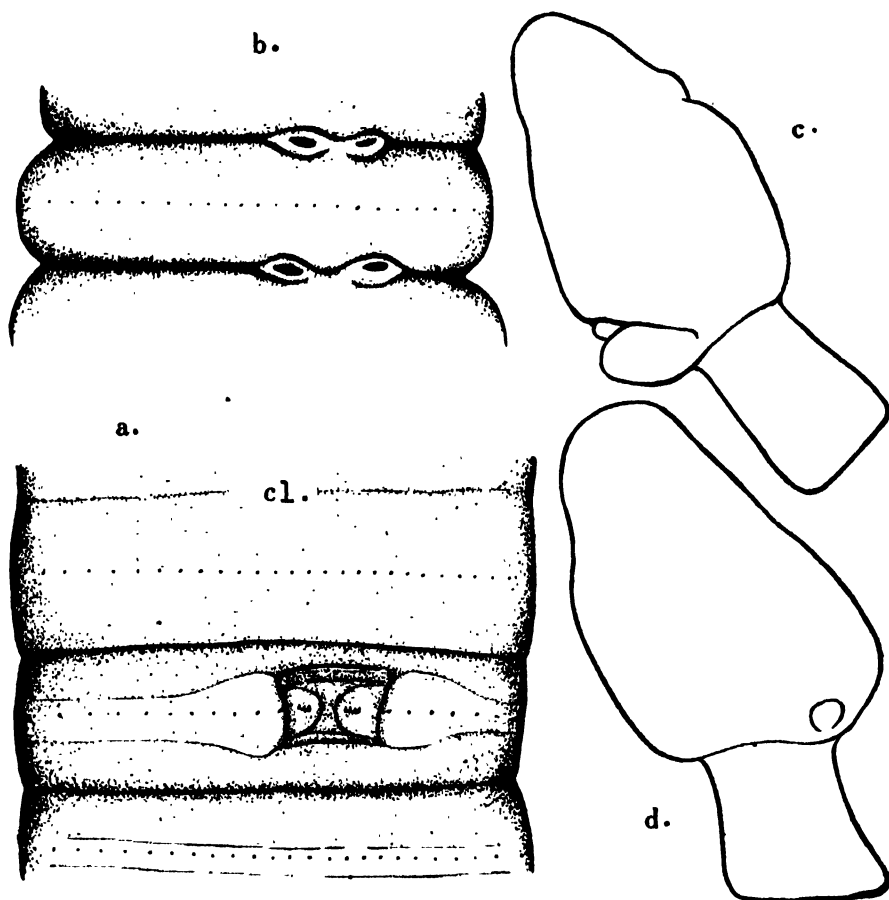
Fig. 1. *Perionyx excavatus* E. PERRIER. a. Ventral view of XVII–XIX, × ca. 33.5; cl. clitellum. b. Spermathecal pore aspect, × ca. 18.9. c & d. Spermathecae with knob-like swellings, × ca. 33.5.

anteriorly to 17/18 nor posteriorly to 18/19; both anterior and posterior margins are distinct, but the lateral margins are indistinct. The major portion of the depression is occupied by two papillae which are closely placed in the midventral line. On each of these papillae are found 3–4 penial setae. (As there is only a single specimen at hand, exact measurements of them were not made.)

Female pore is single, presetal on XIV, about half way between the setal line and 13/14.

Spermathecal pores (Fig. 1, b) are midventral, closely paired in 7/8 and 8/9. Each pore is a largely opened slit with a slightly elevated rim which appears whitish.

Nephridiopores are located on the anterior margin of the segments and are arranged in the same longitudinal line.

Internal anatomy:

No septa specially thickened. A vestigeal gizzard in VI. Intestine begins to swell in XIII. Intestine in XIII is a little more thick-walled than the rest and appears whitish. Last hearts in XII, small in calibre. Dorsal vessel in the region of about XVIII–XXVIII fairly enlarged.

Testes and funnels are free, in X and XI. Seminal vesicles are two pairs in XI and XII, large; as they have collapsed, their original features could not be satisfactorily examined.

Prostate gland small, confined to only XVIII, but slightly pushing 18/19 posteriorly; greyish, compact, thickly cushion-like, sessile on parietes, poorly incised. Duct short, thin and straight.

Ovaries large, in XIII.

Spermathecae are large, erect and with the ental ends in contact transversely over the dorsal blood vessel. Ampulla is ovoidal with narrower ental portion, containing much whitish mass; in two of the examined three cases, one or two knob-like swellings were found on the proximal surface (Fig. 1, c & d). (From the structure and the relative position, these swellings are not considered to be any kind of vestigial diverticula.) Duct short but thick and musculated. Diverticulum absent.

Occurrence: Shinchiku, North Formosa.

Distribution: E. Himalayas, W. Himalayas, S. India, S-W. India, Ceylon, Bengal, Burma, Little Andaman Island, Siam, Cochin-China, Java, Borneo, Sumatra, Sangi Island, Polu Nias, Mentawai Island, Madagascar, Réunion Island, Philippines, Formosa.

RELATIVE GROWTH OF *MERETRIX MERETRIX* AND ITS LOCAL VARIATION, AS SHOWN BY EXPERIMENTS IN REARING

By

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(With five Figures)

(Received July 19, 1938)

In many cases, the existence of local variations in the structure etc. of animals has long been known. To account for such variations many environmental factors have been considered, *e. g.* temperature, light, chemical conditions of the medium, conditions of food supply, and other physical, chemical, and biological conditions of environment. In the case of the marine mollusc, *Meretrix meretrix,* the present writer has found the local variations in the shell, and has pointed out the effects of the environmental conditions, mainly of temperature (HAMAI, 1934), and of the physical nature of the sand in which the clams live, and of the salinity and other chemical conditions of sea water (HAMAI, 1935 a). And, this fact has been further confirmed by the seasonal changes in the clam with the changes of temperature (HAMAI, 1935 b). For the purposes of establishing these facts more completely, experiments based on the transference of the mollusc from one locality to another have been carried out.

In the cases mentioned above, the existence of local variations has been established as regards the relative growth. In the studies of relative growth, the allometric equation has ordinarily been applied by many investigators. But, since in applying this equation the time factor is eliminated, the conclusions deduced with regard to the breaking point are often misleading, and the relationship between the relative growth and the variation in size is apt to be vague. Some criticisms and the establishment of the relationship between relative growth and variation in size have also been attempted in this paper, based on the data of this investigation. The relationship between relative growth and variability together with criticisms on these points are dealt with in Part I, and the local variation in Part II.

Before proceeding further, the present writer wishes to express his

I. HAMAI

sincere thanks to Prof. E. NOMURA for his kind guidance, and also to the
Fishery Experiment Stations in Hirosima, Watanoha, Tiba, Kawagoe,
Saizyô, and Okinohata for their assistances in this investigation, and further
he is indebted fo Mr. T. SEKI for some of the specimens.

MATERIAL AND METHOD

The clams collected at Nagahama in Watanoha and at Ômagarihama,
both in Miyagi Prefecture, were sent to Samukawa, Tiba Prefecture, to

TABLE 1.

Exp. No.	Localities in which the specimens were collected	Date of collection	Localities to which the specimens were transferred	Date of transference	End of rearing period	No. of specimens	No. of cages	No. of survivals at the end of rearing period
1	Nagahama	July 2, 1935	Samukawa	July 5, 1935	July 10, 1936	100	2	14
2	,,	July 2, 1935	Kawagoe	July 6, 1935	July 8, 1936	100	2	0 ⎫
3	,,	Aug. 13, 1935	,,	Aug. 16, 1935	,,	100	2	0 ⎬ *
4	,,	Sept. 20, 1935	,,	Sept. 23, 1935	,,	100	2	15 ⎭
5	,,	July 15, 1935	Kusatu	July 20, 1935	,,	66	1	31
6	,,	Aug. 13, 1935	,,	Aug. 17, 1935	,,	50	1	10
7	,,	July 2, 1935	Okinohata	July 10, 1935	July 13, 1936	100	2(1)	1 ⎫
8	Ômagari-hama	Dec. 11, 1935	,,	Dec. 14, 1935	,,	about 100	1	30 ⎬ **

* Almost all the clams which had been transferred were dead by July 13, on
account of the heavy rain-fall owing to which the salinity fell nearly to that of fresh
water. A second experiment was therefore undertaken on August 16. In this case
too, the rain-fall lasted and in consequence the specimens were all dead by September
13. Again, a third experiment was made on September 23. 100 specimens were
reared in the cages, being divided equally into two groups. In this last experiment,
the number of survivals at the time of inspection from time to time were as follows:
47 individuals on October 9, 1935, and 20 on January 14, 1936.

** Inspection of the cages on September 30, 1935, showed that about half the
specimens were dead on account of the attachment of numerous oyster spats, for the
sand in the cage was washed out by the current and the clams were exposed not
being covered by the sand. Then, the survivals of the specimens in the two cages
were all put into one cage together, and the sand was again replaced. Further, the
specimens at Ômagarihama were reared in another cage from December 14, 1935.

Kawagoe, Mie Prefecture, to Kusatu, Hirosima Prefecture, and to Okino-hata, Hukuoka Prefecture, and were reared in wire-netting cages placed on the sea bottom in the respective locality. The wire-netting cage measured 50 cm. cube with a 1 cm. mesh. It was fixed in the sand, the upper part 10–15 cm. in measurement being exposed to the sea water and the lower part being embedded in the sand. Each cage contained 50 specimens. The records of the beginning and of the end of the rearing process and other data are shown in Table 1.

Reverse experiments were carried out. The specimens collected at Kusatu, Saizyô, and Kawasaki were reared in Mangoku-ura at Watanoha. As the control experiment, the specimens from Nagahama at Watanoha were also reared in the same locality (Table 2). The rearing stations in all these localities lie in the intertidal zone.

TABLE 2.

Exp. No.	Localities in which the specimens were collected	Date of collection	Date of trans-ference	End of rearing period	No. of specimens	No. of cage	No. of survivals at the end of rear-ing period
9	Kusatu	July 24, 1935	Aug. 4, 1935	July 21, 1936	100	2	99
10**	,,	,,	,,	,,	50	1	48
11	Kawasaki	Aug. 3, 1935	Aug. 4, 1935	,,	50 (49)*	1	45
12	Saizyô	—	Dec. 9, 1935	,,	100(45)* (46)*	1 1	0 5
13	Nagahama	Aug. 13, 1935	Aug. 13, 1935	,,	103 (102)*	2	30

* Some specimens were lost at the time of placing them in the cage.
** These specimens were all of the same age and were those bred in the summer season of 1934. These specimens have been dealt with in Part 1.

During the rearing period, sea-weeds and other organisms which had become attached to the exposed part of the cages were removed from time to time, the cages were always kept clean, and the water circulating through them was kept in good condition.

The method of measurement and calculation is the same as that used in the present writer's previous work (HAMAI, 1934). The results of measurement were classified in several groups, logarithmically, and these were adopted in the allometric equation, which was applied throughout

the whole range of size, approximately. The calculation is subject to the method of least squares.

PART I.
RELATIONSHIP BETWEEN THE RELATIVE GROWTH AND THE VARIATION IN SIZE

Results. 50 specimens bred in Kusatu were reared in Mangoku-ura, all the specimens being of the same age. When the height and depth of the shell are plotted against its length, each age-group seems, respectively, to follow a straight line (Tables 3 and 4 ; Fig. 1).

TABLE 3.

Measurements on July 24, 1935.

Number of specimens	Class of length	Mean length	Mean height	Mean depth
1	1.91 - 2.00	1.950	1.730	0.930
8	2.01 - 2.10	2.076	1.800	1.019
30	2.11 - 2.20	2.163	1.869	1.050
7	2.21 - 2.30	2.253	1.943	1.104
3	2.31 - 2.40	2.340	1.987	1.117
1	2.41 - 2.50	2.420	2.010	1.140
50		2.173 ± 0.0076	1.875 ± 0.0053	1.056 ± 0.0034

TABLE 4.

Measurements on July 21, 1936.

Number of specimens	Class of length	Mean length	Mean height	Mean depth
2	2.61 - 2.70	2.655	2.140	1.265
1	2.71 - 2.80	2.800	2.310	1.400
0	2.81 - 2.90	-- -	-- --	---
2	2.91 - 3.00	2.935	2.460	1.495
1	3.01 - 3.10	3.010	2.470	1.440
1	3.11 - 3.20	3.160	2.650	1.500
1	3.21 - 3.30	3.300	2.800	1.650
3	3.31 - 3.40	3.353	2.847	1.703
3	3.41 - 3.50	3.477	2.887	1.767
7	3.51 - 3.60	3.544	2.956	1.807
14	3.61 - 3.70	3.651	3.018	1.896
6	3.71 - 3.80	3.747	3.087	1.915
4	3.81 - 3.90	3.835	3.220	1.965
3	3.91 - 4.00	3.957	3.207	1.960
48		3.532 ± 0.031	2.929 ± 0.025	1.799 ± 0.018

Also, the log/log plot of the same data seems to be expressed by a straight line, *i.e.* the height/length and the depth/length relations can be expressed by the simple allometric equation ; but, also in this case, the group of each age seems, respectively, to conform to a different equation (Fig. 2). Then, as to the equation expressing the direction of variation, the question arises as to superiority in suitability between the forms $y = ax + c$ and $y = bx^a$. This problem will be discussed in a later section.

The results of growth are shown in Tables 3 and 4. The growth during one year, from July 24, 1935, to July 21, 1936, was 1.359 cm. in length, 1.054 cm. in height, and 0.743 cm. in depth, as to one year old specimens. The size-distribution of the length increased during one year from C.V.=3.7 to 8.9, that of the height from 3.0 to 8.9, and that of the depth from 3.3 to 10.1.

Discussion. The variation in most animals is usually subject to the
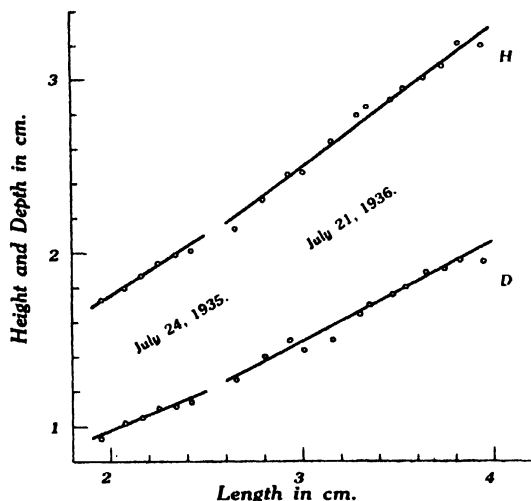
Fig. 1. Points are plotted from Tables 3 and 4. The straight lines express $y = (\sigma_y/\sigma_r)x + y_1$.
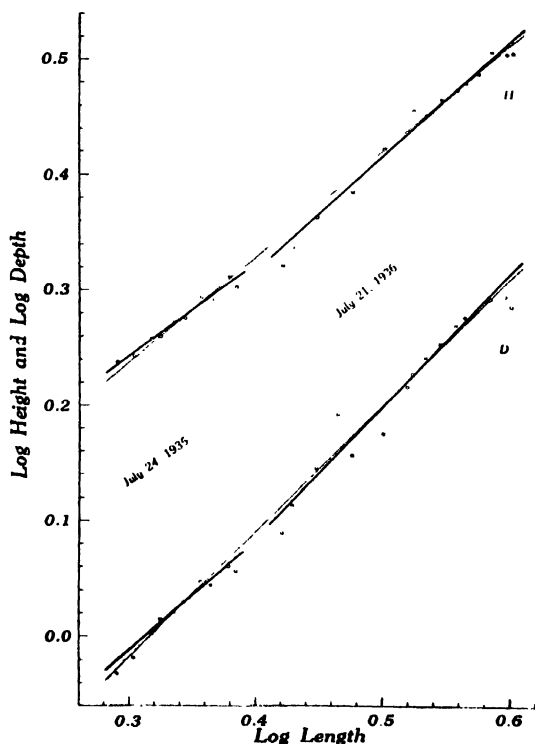
Fig. 2. Log/log plot of the height and depth against the length of the shell. The straight lines express the allometric equations. The light lines express the relative growth.

normal frequency curve. The variation in size in the case of *Meretrix meretrix* may also be expected to follow this curve. As a matter of fact,
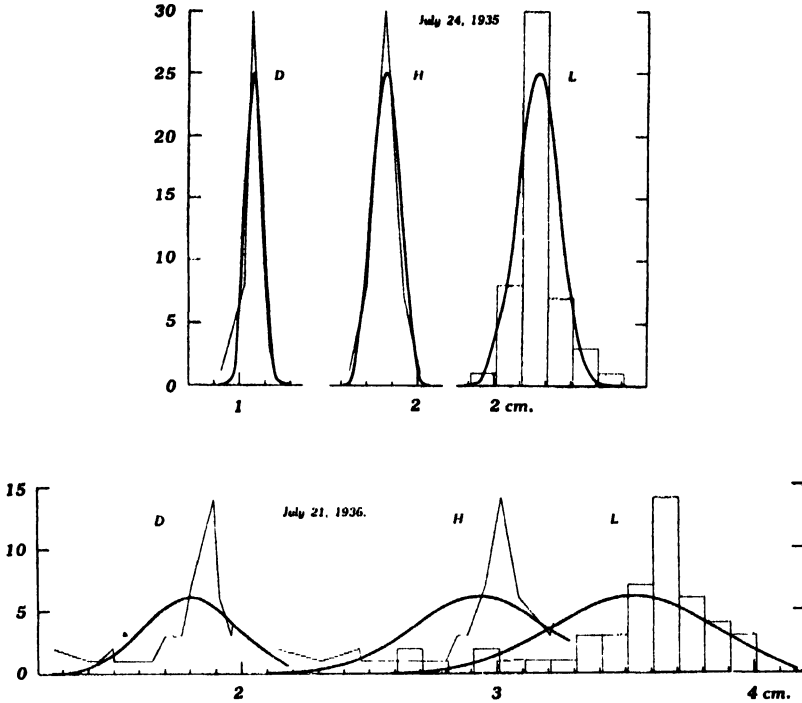
Fig. 3. Distribution of size. The smooth curves represent the normal frequency curves.

the specimens examined on July 24, 1935, were approximately subject to the normal equation; however, those examined on July 21, 1936, showed a slight asymmetrical distribution and seemed not to be subject to the normal equation (Fig. 3). The calculated values of the constants in the normal equation, $f = f_0 e^{-\frac{(x-x_0)^2}{2\sigma^2}}$, where f is the frequency, f_0 the maximum frequency at the mean size, σ the standard deviation, x the size, x_0 the mean size, and e the base of the natural logarithm, are shown in Table 5.

If the normal equation may be theoretically applied, and the coefficient of correlation is 1, — *i. e.* the complete correlation is expressed, — the variation in the two dimensions, x and y, is, respectively, represented by the formulae ; —

TABLE 5.

Constants in the normal equation.

Date of measurement	Dimension	f_0	x_0	σ
July 24, 1935	length	25.0	2.173	0.079684
	height	,,	1.875	0.055737
	depth	,,	1.056	0.035286
July 21, 1936	length	6.1	3.532	0.31433
	height	,,	2.929	0.25961
	depth	,,	1.799	0.18180

$$f = f_0 e^{-\frac{(x - x_0)^2}{2\sigma_x^2}}$$

$$f = f_0 e^{-\frac{(y - y_0)^2}{2\sigma_y^2}}$$

From these formulae the equation for a straight line is derived in regard to these two dimensions; *viz.*

$$y - y_0 = (\sigma_y/\sigma_x)(x - x_0)$$

or $\qquad y = (\sigma_y/\sigma_x)x + y_1 \qquad\qquad y_1 = y_0 - (\sigma_y/\sigma_x)x_0.$

This equation coincides with that derived directly from the data of measurement of two dimensions by the method of least squares. As tested in practice, it was shown that the correlation coefficients between the length and height, and between the length and depth, are approximately 1 (Table

TABLE 6.

Correlation coefficient.

Date of measurement	Height/length	Depth/length
July 24, 1935	0.990	0.973
July 21, 1936	0.971	0.984

TABLE 7.

Constants in the equation, $y = (\sigma_y/\sigma_x)x + y_1$.

Date of measurement	Height/length relation		Depth/length relation	
	$\sigma_y/\sigma_x \pm$ P. E.	$y_1 \pm$ P. E.	$\sigma_y/\sigma_x \pm$ P. E.	$y_1 \pm$ P. E.
July 24, 1935	0.69948 ± 0.00957	0.355 ± 0.021	0.44282 ± 0.00997	0.094 ± 0.022
July 21, 1936	0.82592 ± 0.01086	0.012 ± 0.039	0.57837 ± 0.01016	-0.244 ± 0.036

6). The application of this equation to the present data seems, therefore, to be not inconsistent, but to have merely a theoretical meaning. The practical use of this equation is shown in Table 7.

The application of the allometric equation to these data is also reliable. That is, when the data are plotted logarithmically on both axes, it is not inconsistent to apply a straight line, but the deviations from the theoretical values are extremely small (Table 8). This equation has been successfully used in expressing the direction of variation in *Cypraeidae* (HAMAI, 1938).

TABLE   8.

Application of the allometric equation.

Date of measurement	Height/length relation		Depth/length relation	
	$\alpha \pm$ P. E.	$\log b \pm$ P. E.	$\alpha \pm$ P. E.	$\log b \pm$ P. E.
July 24, 1935	0.8100 ± 0.0154	0.00003 ± 0.00519	0.9466 ± 0.0198	$\bar{1}.70451 \pm 0.00669$
July 21, 1936	0.9990 ± 0.0154	$\bar{1}.91916 \pm 0.00843$	1.1400 ± 0.0225	$\bar{1}.62972 \pm 0.01234$

From these two equations the theoretical values of y have been calculated, and the differences between the observed and theoretical values have been compared in the case of both equations (Table 9). It was found,

TABLE   9.

Comparison of fd^2/N between the two equations.

Equation	July 24, 1935		July 21, 1936	
	Height	Depth	Height	Depth
$y = bx^a$	0.000111	0.000669	0.00159	0.00135
$y = (\sigma_y/\sigma_x)x + y_1$	0.000064	0.000044	0.00118	0.00103

as the result of the comparison, that, empirically, the fitness of the curve is rather greater in the straight line than in the allometric equation. After all, the equation of the straight line is superior to the allometric equation in the point of theoretical foundation, as the expression of the variation of size. It has been already explained that the allometric equation expresses an empirical approximation in the relative growth (HAMAI, 1937 ; LUMER, 1937). In expressing the trend of variation, this equation is also an empirical approximation rather than a theoretical one.

TEISSIER (1937) has shown that the constants of the allometric equa-

tion are, in practical application, approximately given by the following relations : —

$$\alpha = V_y/V_x = (\sigma_y/\sigma_x)(x_0/y_0) \qquad \text{and} \qquad b = y_0/x_0$$

where V_x and V_y are the coefficient of variation of x and $\dot{y}$, respectively, σ_x and σ_y are, respectively, the standard deviation of x and y, and x_0 and y_0 represent the mean values of x and y respectively. These relations have been re-examined by comparing them with the data given here, but they are not necessarily valid in these data, as shown in Table 10.

TABLE 10.

Comparison between the actual calculation and TEISSIER's *derivation, with regard to the constants in the allometric equation.*

Date of measurement	Height/length relation				Depth/length relation			
	α	V_y/V_x	b	y_0/x_0	α	V_y/V_x	b	y_0/x_0
July 24, 1935	0.810	0.811	1.000	0.863	0.947	0.911	0.506	0.486
July 21, 1936	0.999	0.996	0.830	0.829	1.140	1.136	0.426	0.509

Accordingly, TEISSIER's derivation of the constants in the allometric equation has no practical validity, and it is also difficult to derive these relations theoretically. Thus, the application of the allometric equation to the direction of variation is merely an empirical approximation.

Without reference to the superiority of the method of expressing the direction of variation, it is quite clear that the direction of variation changes with age, and that its differences are statistically significant (Tables 7 and 8).

If the specimens, which include those of every age, were collected at a certain time, and their measurements were plotted on the logarithmic grid, one or more breaking points would appear. This fact may be fairly presumed from the fact that in the data here given the directions of variation are, respectively, different in each age-group from those of the other groups. In such a case the existence of the breaking points is apt to cause investigators of the relative growth to overlook the direction of variation and to be misled regarding the true relative growth. As a matter of fact, the true relative growth in this case is to be measured by considering the whole as one curve or by considering the mean values of all ages as successive points of a whole series. Thus, in this case of *Meretrix meretrix*, the two age groups ought to be put together, and

then the whole group must be analysed by the method of relative growth. If the data are analysed by the method of approximation, *viz.* the application of the allometric equation, the equilibrium constants and the initial indices will be as given in Table 11. That is, the height/length relation shows a negative allometry, and the depth/length a positive. This tendency of relative growth is general in the case of *Meretrix meretrix.*

TABLE 11.

Constant	Height/length relation	Depth/length relation
a	0.921 ± 0.003	1.095 ± 0.005
b	0.917 ± 0.003	0.451 ± 0.002

PAULIAN (1936) studied the relative growth of the scorpion, *Buthus occitanus*, and observed two phases in the growth, *i.e.* the first phase covering the first two ages, and the second the later ages, which correspond to the third summit of his frequency curves. The latter phase is very vague, because the ages are not clearly distinguished, and so this "third summit" is apt to be confused with the direction of variation of the later age-group.

ANDERSON, LUMER and ZUPANCIC (1937) have observed the variability of every instar in *Daphnia pulex*, and found a definite relationship, *viz.* that the relative variability in body size, as measured by the coefficient of variation of total length, is, roughly, directly proportional to the logarithm of the growth rate. They have tentatively concluded that the observed trend in variability in body size with growth, and its relation to the growth rate, are explicable largely in terms of the action of environmental factors, and perhaps also, in part, in terms of RAHN's (1932) theory. The frequency curves of various sizes in the case of *Meretrix meretrix* become flatter relatively to the increase of age, and then, more asymmetrical. These effects must ba analysed from the point of view of the physico-chemical reactions with respect to growth and of the environmental factors.

PART II.

LOCAL VARIATION IN *MERETRIX MERETRIX*

The change of the form of the shell is measured by the difference in the values of a and b in the allometric equation, because it is affected

by the mode of growth, *viz.* the relative growth. The values of these constants, at the beginning and at the end of the rearing period, however, do not in general show significant differences. But only in the depth/length relation in Experiment 9, a very significant difference occurred; *i.e.* α

TABLE 12.

Exp. no.	Conditions at the beginning of rearing period		Conditions at the end of rearing period	
	$\alpha \pm$ P. E.	$b \pm$ P. E.	$\alpha \pm$ P. E.	$b \pm$ P. E.
1	0.964 ± 0.003 *1.216 ± 0.004*	0.837 ± 0.002 *0.335 ± 0.001*	0.956 ± 0.023 *1.188 ± 0.038*	0.838 ± 0.022 *0.348 ± 0.015*
4	—	—	0.908 ± 0.024 *1.007 ± 0.039*	0.889 ± 0.021 *0.427 ± 0.016*
5	0.963 ± 0.002 *1.213 ± 0.008*	0.835 ± 0.002 *0.331 ± 0.002*	0.972 ± 0.012 *1.210 ± 0.019*	0.826 ± 0.009 *0.332 ± 0.006*
6	—	—	0.962 ± 0.011 *1.256 ± 0.030*	0.837 ± 0.010 *0.322 ± 0.010*
8	—	—	0.909 ± 0.010 *0.895 ± 0.013*	0.924 ± 0.013 *0.559 ± 0.010*
9	0.921 ± 0.003 *1.004 ± 0.005*	0.916 ± 0.003 *0.483 ± 0.002*	0.923 ± 0.005 *0.972 ± 0.008*	0.907 ± 0.005 *0.517 ± 0.005*
11	0.910 ± 0.015 *0.951 ± 0.018*	0.914 ± 0.020 *0.520 ± 0.014*	0.872 ± 0.018 *0.896 ± 0.018*	0.964 ± 0.028 *0.572 ± 0.016*
12	0.916 ± 0.005 *1.086 ± 0.008*	0.930 ± 0.006 *0.440 ± 0.005*	—	—
13	0.947 ± 0.004 *1.178 ± 0.008*	0.844 ± 0.003 *0.342 ± 0.002*	0.950 ± 0.011 *1.171 ± 0.023*	0.843 ± 0.011 *0.336 ± 0.009*

Numerals in common type express the height/length relation and those in Italics the depth/length.

TABLE 13.

Changes of a_m.

Exp. no.	Beginning of Experiment		End of Experiment	
	H/L	D/L	H/L	D/L
1	0.862	0.391	0.863	0.411
4	—	—	0.871	0.414
5	0.857	0.379	0.861	0.391
6	—	—	0.865	0.404
8	—	—	0.898	0.459
9	0.908	0.468	0.899	0.475
11	0.887	0.457	0.879	0.454
12	0.912	0.468	—	—
13	0.855	0.381	0.863	0.392

changes from 1.004 ± 0.005 to 0.972 ± 0.008 and b from 0.483 ± 0.002 to 0.517 ± 0.005. And the survival ratio of the specimens bread in Kusatu is much greater than those in other localities. This fact suggests that the change of form depends upon the adaptability in each case to the respective environment. Further, it is suggested that the general unchangeableness of α and b, with the exception of the specimens from Kusatu, proves that the local type is almost invariably stable.

Some years ago, the present writer studied the local variation in *Meretrix meretrix*, and determined the local constant by means of the equation, $y = a_m x^{\alpha_0}$*, where x is the length, y the height or depth, of the shell, α_0 represents the genetic constant of the species, *viz.* the mean value of α in various localities, and a_m is the local constant. In that case, α_0 was determined as 1.04 in the depth/length relation and as 0.93 in the height/length relation (HAMAI, 1934). The results of the rearing experiments have accordingly been calculated by means of the genetic constants previously dertermined (Table 13).

At the beginning of the rearing experiment each local constant varies with the temperature of environment, and this fact coincided with the results of previous observation (HAMAI, 1934). As shown in Table 13, in the specimens transferred from Nagahama at Watanoha to Samukawa (Exp. 1), to Kusatu (Exp. 5 and 6), and to Mangoku-ura at Watanoha (Exp. 13), and, perhaps, to Kawagoe, the local constants in both the height/length and depth-length relations increased slightly, and in those transferred from Kusatu and Kawasaki to Mangoku-

Fig. 4. Relation between the local constants and surface temperature of sea-water. ● a_m, ○ b.

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*In this case, this equation has been expressed as $y = a_m x^b$, but in the present case, α_0, being subject to the allometric equation, is used in place of b.

ura (Exp. 9 and 11), they slightly decreased with the exception of the depth/length relation in Exp. 9: *i. e.* in general, when the clams were transferred from Nagahama to other localities, the local constants slightly increased, and in the case of the reverse transference, they decreased slightly.   If a relationship between the local constants and temperature exists, and if it is true that the higher the temperature, the greater the increase in the local constant, the increases in a_m are perhaps accounted for in the specimens transferred from colder localities, *e. g.* Nagahama and Ômagarihama, to the localities of higher temperature, *e. g.* Samukawa, Kusatu and Okinohata, and in those from the higher temperature to the lower, decreases in a_m are probable.   But these increases and decreases were, actually, very slight, and this fact suggests that it is relatively difficult to change the comparatively stable local type during such a short period as one year or less than one year.

<div align="center">

TABLE   14.

Annual average (from July, 1935, to June, 1936)
surface temperature of sea-water.

</div>

Locality	Temperature (°C)
Mangoku-ura, Miyagi Prefecture	12.5
Samukawa, Tiba Prefecture	16.4*
Kawagoe, Mie Prefecture	16.2
Kusatu, Hirosima Prefecture	17.3
Okinohata, Hukuoka Prefecture	16.7
Saizyô, Ehime Prefecture	17.7*

* These figures are taken from HAMAI's paper (1934). The temperature in Kawasaki seems roughly equal to that in Samukawa, which also faces Tôkyô Bay.

In the case where the temperature effect is considered, the seasonal change of temperature must be taken into account.   The seasonal changes of temperature in three localities, Mangoku-ura, Kusatu and Kawagoe, are roughly parallel, but the change in Okinohata is different from those in these localities, *i. e.* the difference between the maximum and minimum temperature is greater than those in other localities.   In Exp. 8, in which the specimens were transferred from Nagahama to Okinohata, at the end of the experiment a_m were greatest in the specimens from Nagahama

and Ômagarihama. This fact suggests that this effect is the result of the change of temperature.
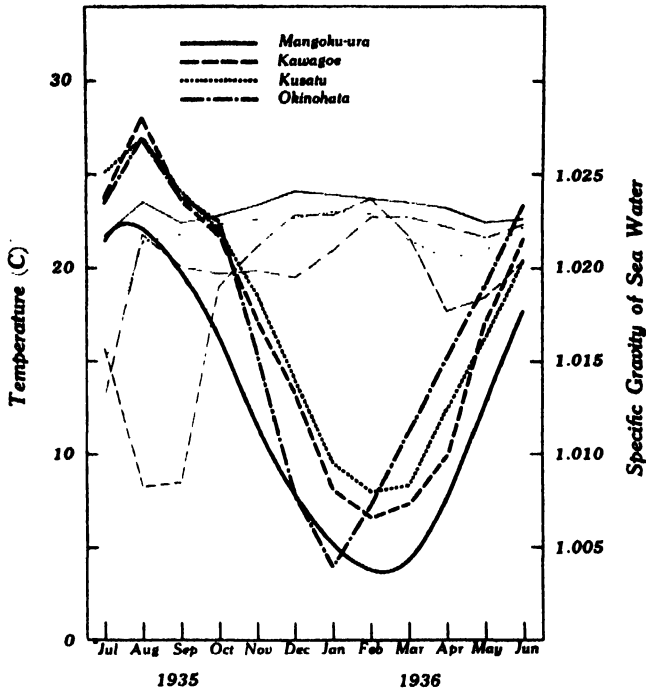
Fig. 5. Seasonal change of temperature and specific gravity of sea-water. Heavier lines represent temperature and lighter lines specific gravity. The records at Kawagoe, Kusatu and Okinohata are those of the sea-bottom, and those in Mangoku-ura those of the surface water.

The seasonal change of salinity is determined by that of the specific gravity. The form of the change of specific gravity is similar in every locality, i. e. in the summer season it is lower and in the winter season it is higher. The change in Kawagoe is the greatest in the four localities, and the low specific gravity in the summer of 1935 in Kawagoe appears to have caused the high mortality.

The changes of the local constants as the result of the transference from Nagahama to Mangoku-ura, the temperature perhaps being equal in both localities, can be accounted for by other factors. It has been previously observed that the elongated type of *Meretrix meretrix* grows on a sandy shore facing the open sea and the roundish type in a calm sea

with a slightly muddy bottom (HAMAI, 1935 a).   To compare the condi-
tions of environment at Nagahama with those at Mangoku-ura, the former
is a snady shore facing the open sea and the latter a calm sea with a
slightly muddy bottom.   The specimens at Nagahama therefore have to
be of an elongated type, which has smaller local constants, and those at
Mangoku-ura of a roundish type, which has greater local constants.   Thus.
the increase in the local constants are explicable in the case of the trans-
ference from Nagahama to Mangoku-ura.

Only in one case, that of Exp. 9, did the depth/length relation show
a reverse result, *i. e.* the local constant slightly increased instead of
decreasing after the transference from the higher temperature to the lower.
This fact cannot be accounted for except as the result of other factors
which are still undiscovered.

At any rate, these changes in the local constants are infinitesimal
during such a short period as one year or less than one year, and; then,
merely suggest a modifiability of the local type.

SUMMARY

By means of rearing experiments after the transference of the specimens
from one locality to another, the relationship between the variability and
the relative growth in *Meretrix meretrix* and its local variation have been
discussed, and are summarized as follows : —

1) The variability of the length increases during one year from C. V.
=3.7 in the first year of growth to 8.9 at the end of the second year,
that of the height from 3.0 to 8.9 and that of the depth from 3.3 to 10.1.

2) The trend of variability is more practically and theoretically ex-
pressed by the linear relation, $y = (\sigma_y/\sigma_x)x + y_1$, where x and y are dimen-
sions, σ_x and σ_y the standard deviations of x and y respectively, and y_1 a
constant, rather than by the allometric equation.

3) The relative growth during one year from the end of the first year
to the end of the second year (from July 24, 1935, to July 21, 1936) is
expressed approximately by the allometric equation (Table 11) in the
conditions of rearing after transference from Kusatu to Mangoku-ura at
Watanoha.

4) The relative growth must be considered with respect to the age
quite apart from the trend of the variability.

5) The results of the rearing experiments by means of transference
suggest a definite modifiability of local type, *i. e.* environmental factors

may perhaps modify the local type in a definite manner.

6) The local type of *Meretrix meretrix* is extremely stable.

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STUDIES ON THE GROWTH HORMONES OF PLANTS
IV. FURTHER EXPERIMENTS ON THE PRODUCTION OF GROWTH SUBSTANCE IN ROOT-TIPS.

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INTRODUCTION

The problem of the production of the growth substance in root-tips has been investigated by a number of workers, but the problem of whether the growth substance is produced in the tips themselves, or it is transported and accumulates there still remains unsolved. (As to the literatures in relation to this problem, cf. NAGAO 1936, VAN RAALTE 1937, WENT & THIMANN 1937 etc.) The present writer (1936) has shown that the amount of growth substance, diffused from 2 mm root-tips of *Avena sativa* into dextrose agar within an hour, is about twice as great as the amount obtainable by direct extraction with chloroform, and that the root-tips continue to give the growth substance into the agar blocks for at least 6 hours. From the above results, and, taking into consideration also the fact that the transport of the growth substance in the root-tips takes place far more easily basipetally than acropetally, the present writer has concluded that the growth substance is actually produced in the root-tip. The same conclusion has been reached by BOYSEN JENSEN (1936) and VAN RAALTE (1936, 1937).

FIEDLER (1936), however, could not find the growth substance in the isolated root-tips of *Zea Mays* and of *Pisum sativum*, which continued to grow in a culture medium, and were normally geotropic. He concluded that root-tips when cultured produce no growth substance, and adopted THIMANN's view (1934) that the growth substance in root-tips is transported from the seed or shoot.

As opposed to FIEDLER's results, the present writer (1937) has shown that, in the case of *Helianthus annuus*, a considerable amount of growth substance is found in the root-tips when cultured. The present paper reports the results of further experiments on the production of growth substance in isolated root-tips cultured under sterilized conditions.

MATERIALS AND METHODS

Pisum sativum and *Zea Mays* were used as material plants. At first, the seedlings of these plants were cultured under sterilized conditions in a similar way to that described in the previous paper (NAGAO 1937): — The seeds were soaked in tap water for about an hour, then sterilized in about 3% H_2O_2 for 30-60 minutes and washed in sterilized tap water. These seeds were allowed to germinate in test tubes on sterilized sawdust at about 25°C. Later, however, the method of cultivation of the seedlings was altered in the following way to obtain a larger number of seedlings more easily. The seeds were soaked in tap water under diminished pressure for 20-30 minutes, then sterilized in 0.1% $HgCl_2$ under diminished pressure for 20-30 minutes and afterwards washed in sterilized tap water. These seeds were allowed to germinate on sterilized 1% agar in large Petri dishes, 18 cm in diameter. (About 70 seeds in each dish.)

After three or four days the roots of the seedlings became several cms in length. The root-tips, 1 cm long, were cut off, and cultured under sterilized conditions in test tubes or Petri dishes. The cultures were kept in a dark place, but were brought into the light from time to time to inspect the state of the growth.

The culture medium was prepared after WHITE (1934) or BONNER and ADDICOTT (1937). The composition is given in Table 1.

TABLE 1.

Composition of Medium Used for Cultivation of Excised Roots.

Substance	WHITE's medium mg. per litre of dist. water	BONNER & ADDICOTT's medium mg. per litre of dist. water
$Ca(NO_3)_2 \cdot 4H_2O$	142	236
$MgSO_4 \cdot 7H_2O$	74	36
KNO_3	81	81
KCl	65	65
KH_2PO_4	12	12
$Fe_2(SO_4)_3$	3	2
Glucose	20,000	—
Sucrose	—	40,000

To the above 10 grams of agar was added. Other substances such as extract of yeast or vitamin B_1 were not added.

The growth substance was tested by the modified WENT's *Avena* technique (1928) in the usual way. (Cf. NAGAO 1937, 1936.)

EXPERIMENTS

1. The Occurrence of Growth Substance in Isolated Roots Grown under Sterilized Conditions.

In the previous paper (1937) the present writer reported that, in the case of *Helianthus annuus*, the tips of isolated roots cultivated under sterilized conditions give a considerable amount of growth substance. This fact does not agree with the results obtained by FIEDLER (1936) after using *Zea Mays* and *Pisum sativum* as material plants. To settle the question of whether, the above disagreement is caused by the difference in materials or not, the experiments were made, the same species of plants as those used by FIEDLER being experimented on.

Root-tips were cultured in test-tubes containing about 15 ccm of the nutrient medium. A single root-tip was placed in each test tube. The excised root-tips continued to grow normally and geotropically downwards into the space between the agar and the wall of the test tubes. Tips about 2 mm in length of these roots were cut off and the growth substance was extracted by BOYSEN JENSEN's dextrose-agar-method (1933). The volume of the agar blocks was 2 cmm ($2 \times 1 \times 1$ mm). A single tip was placed on each agar block for 4 hours. Results are shown in Table 2.

TABLE 2.

Growth Substance in Isolated Root-Tips (Mean of 6 plants)

Date	Duration of culture (days)	Culture medium	Average length of roots used (mm)	*Avena* curvature (°)
		Pisum sativum		
2. XI. 37.	2[a]	WHITE	17.6	12.6±1.1[d]
3. XI. 37.	2[a]	WHITE	18.5	22.6±1.6
5. XI. 37.	4[a]	WHITE[c]	27.8	12.0±1.1[d]
19. I. 38.	4[b]	BONNER & ADDICOTT	38.1	16.3±1.6
		Zea Mays		
10. II. 38.	4[b]	WHITE	39.3	15.0±1.3
10. III. 38.	12[b,e]	WHITE	185	8.3±1.0

a) The temperature during cultivation was about 21°C.
b) The temperature during cultivation was about 25°C.
c) Agar was not added.
d) Mean of 4 plants.
e) Large test tubes containing about 45 ccm of medium were used.

Table 2 shows that, in the case of *Pisum sativum* and of *Zea Mays* also, the growth substance is found to a considerable amount in the tips of the isolated roots growing in test tubes.

2. The Occurrence of Growth Substance in Root-Tips in Relation to the Root Growth.

In the present experiment, the cultivation of the root-tips of *Pisum sativum* was made in Petri dishes, containing about 60 ccm of BONNER and ADDICOTT's nutrient agar or of plain agar, at about 25 ˚C. 6 tips were placed in each dish.

The isolated roots kept on plain agar practically ceased to grow within 2 days, while those on nutrient agar continued to elongate. (They, however, did not penetrate into the agar.) The growth substance in tips about 2 mm in length of those roots was extracted by BOYSEN JENSEN's method in the same way to that described in the above section. In tips kept on plain agar, no growth substance was detectable even one day after, this agreeing with FIEDLER's result. In tips cultured on nutrient agar, however, the growth substance was found to be as usual. (Tab. 3.)

One half of the isolated roots, kept on plain agar for 2 days, and proved to have no detectable growth substance, was then transferred to nutrient agar, while the rest remained, but on new plain agar; the root length and amount of growth substance were then measured every day. Results are shown in Table 3.

TABLE   3.

Growth Substance in Root-Tips in Relation to Root Growth
(Mean of 6 plants).

Exp. No.	Date (1938)	Culture		Root length (mm)	*Avena* curvature (˚)
	17. III.	1 day on P. A.[1]		12.3	—*
		1 day on N. A.[2]		12.5	6.7±0.8[3]
	18. III.	2 days on P. A.		12.5	—*
		2 days on N. A.		18.9	24.0±1.6
1	19. III.	2 days on P. A.+1 day on P. A.		12.7	—*
		,, +1 day on N. A.		15.6	7.1±1.0[4]
	20. III.	,, +2 days on P. A.		12.4	—*
		,, +2 days on N. A.		19.4	14.8±1.1
	21. III.	,, +3 days on P. A.		12.4	—*
		,, +3 days on N. A.		23.5	22.2±0.6

Exp. No.	Date (1938)	Culture	Root length (mm)	*Avena* curvature (°)
2	3. IV.	1 day on P. A.	12.3	—*
		1 day on N. A.	12.5	10.6±0.9
	4. IV.	2 days on P. A.	12.8	—*
		2 days on N. A.	17.6	21.0±1.5
	5. IV.	2 days on P. A.+1 day on P. A.	12.4	—*
		„ +1 day on N. A.	14.8	6.1±0.4
	6. IV.	„ +2 days on P. A.	12.4	—*
		„ +2 days on N. A.	19.2	13.8±0.7

1) P. A. means plain agar.
2) N. A. means nutrient agar.
3) Mean of 5 plants.
4) Mean of 4 plants.
 * 0° or a slight positive curvature.

The roots on plain agar did not elongate at all, and no growth substance could be detected. On the other hand, the roots transferred to the nutrient agar began to elongate again, in agreement with the result found by MALYSCHEV (1932), and, at the same time, the growth substance also appeared. Further, Table 3 shows that the amount of growth substance apparently increases with the increase of growth rate.

3. Extraction of Growth Substance with Chloroform.

The isolated root-tips of *Pisum sativum* were cultured in the same way as is described above (p. 224). After two days, the growth substance of a certain number of the root-tips was extracted with chloroform by THIMANN's method (1934). In the present experiment, the growth substance in the entire root-tips, not in those 2 mm in length, was tested. The chloroform was evaporated and the residue was dissolved in 0.1 ccm of distilled water. 10 plain agar blocks of 2 cmm ($2 \times 1 \times 1$ mm) were soaked in the solution, and kept over-night in the refrigerator. On the next day, the content of the growth substance was determined.

Results are shown in Table 4. The growth substance was present[a]

[a] It is worth noticing that, in the present experiment, the *Avena* curvature, caused by the growth substance, was restricted to a very short region at the top of the coleoptile, and the curvature increased in the next hour. (Tab. 4.) THIMANN (1935) has found that the *Avena* curvatures, caused by indene-3-acetic acid, are confined to a very short region, and he suggested that this is caused by the slow transport of the substance. (The explanation proposed by THIMANN was supported by AVERY, BURKHOLDER and CREIGHTON (1937) in the case of indole propionic compounds.) In the present experiment, however, the cause of the restricted curvatures was not determined. Further experiments are needed to solve the problem in the present case.

in the roots cultured on the nutrient agar, while none could be detected in those kept on the plain agar.

TABLE 4.

Growth Substance Extracted with Chloroform
(*Mean of 6 plants*).

Date (1938)	No. of roots	Culture medium	*Avena* curvature (°)	
			After 2 hrs.[a]	After about 3 hrs.
7. V.	60	Nutrient agar	4.5	9.8
	60	Plain agar	0 or a slight positive curvature	+4.0
2. VI.	30	Nutrient agar	3.8[b]	6.3[b]
	15	Nutrient agar	2.4[b]	4.8[b]
	30	Plain agar	0 or a slight positive curvature	+6.3

a) Accurate measurement of the curvatures was difficult, because the curvatures were small and restricted to a very short region.

b) The test plant often showed an S-shaped curvature because of a positive curvature in the middle region.

DISCUSSION

The above experiments show (1) that, in the case of *Pisum sativum* and of *Zea Mays* as in the case of *Helianthus annuus* (NAGAO 1937), the growth substance is found, to a considerable amount, in the tips of isolated roots in the culture; (2) that, in the isolated roots of *Pisum sativum*, which were kept on plain agar and ceased to elongate, no " free auxin " (by the diffusion method) and also no " bound auxin " (by the method of extraction with chloroform) are detected; (3) that, when the above roots, showing no detectable growth, are transferred to nutrient agar, they begin to elongate again and, at the same time, the growth substance also appears.

From these facts, it is concluded that the root-tips have the capacity of producing the growth substance.[1] Further, it is clear that there exists a close relationship between the growth substance and the growth of the root, though the present experiments have afforded no clue to the solution of the question whether the presence of growth substance in root-tips is the cause or effect of the root growth.

[1] That the nutrient solution contains no growth substance has been confirmed by THIMANN's method (1934).

Most workers agree in the view that the growth substance plays an important rôle in the growth and tropisms of the root. In spite of FIEDLER's experiments (1936), VAN RAALTE (1937), WENT & THIMANN (1937, p. 150), SCHLENKER (1937, p. 47) and OTTE (1937, p. 58) have suggested the presence or production of a small amount of growth substance, which cannot be detected by the *Avena* test, in the isolated roots in culture. By the present experiments, however, it is proved that the growth substance can be detected by the *Avena* test. On the other hand, GUTTENBERG & SEGELITZ (1938) have shown the presence of growth substance in isolated root-tips of *Zea* in culture, the root being used instead of *Avena* coleoptile as the test material. In their experiment, the extract of cultured roots caused the retardation of elongation and the swelling of the test-roots. This effect of the extract corresponds to that of hetero-auxin, the concentration of which is over 1 in 1 milliard.

SUMMARY

1. The presence of the growth substance in the tips of isolated roots cultured under sterilized conditions is established in the case of *Pisum sativum* and of *Zea Mays.*

2. The isolated roots of *Pisum sativum*, kept on plain agar, practically cease to grow within 2 days, and no growth substance is detectable in their tips.

3. When these roots after having ceased to grow are transferred to nutrient agar, they begin to grow again and, at the same time, the growth substance begins also to appear.

4. From the above facts, it is concluded that the root tips can produce the growth substance, and that the growth substance is closely connected with the growth of roots.

The writer is greatly indebted to Prof. Dr. Y. YAMAGUTI for his kind directions in the course of this work.

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DIE VERBREITUNG DER PURPUR- UND GRÜNBAKTERIEN IN YUMOTO (NIKKÔ) IN BEZIEHUNG ZUR SCHWEFELWASSERSTOFFKONZENTRATION

VON

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(Eingegangen am 1. September 1938)

Im Sommer 1938 habe ich einige Tage bei den Thermen von Yumoto in Nikkô verlebt, um vornehmlich die Art und Weise zu erforschen, wie die dortigen Purpur- und Grünbakterien[1] auf örtliche Schwefelwasserstoffkonzentrationen reagieren.

Analytische Methode : Mit einer Spritze entnommene 10 ccm Wasser werden in 5 bzw. 10 ccm n/100-Jodlösung gegossen, darauf titriert man mit n/100-Thiosulfatlösung zurück.[2] Das ganze Verfahren wurde an Ort und Stelle durchgeführt. Bekanntlich ist das einfache jodometrische Verfahren einigermassen fehlerhaft. Tatsächlich ergeben das dortige Trinkwasser und das Wasser aus der zentralen Partie eines Sees bei den Thermen einen falschen H_2S-Gehalt bis zu 2 mg/l trotz ihrer offensichtlichen Freiheit von diesem Gase.

Der Schwefelwasserstoffgehalt des über die Bakterienkolonien hinfliessenden Thermalwassers ist in untenstehender Tabelle zusammengefasst.

Wie aus dieser Tabelle zu ersehen ist, kann *Chromatium Weissei* bei den Schwefelwasserstoffkonzentrationen, von einer ganz geringfügigen bis zur grösseren Hälfte derjenigen des quellenden Thermalwassers, gedeihen, während die grüne Bakterie *Chlorobium limicola* massenhaft nur dort vorzukommen scheint, wo sie reichlicheren Schwefelwasserstoff zur Verfügung hat.[3] Auffallend ist es, dass der Schwefelrasen unter denjenigen Bedingungen wächst, welche für das quellende Thermalwasser eigentümlich sind.

Weiterhin traf ich ein interessantes Phänomen in einem mit der Kolonie von *Chromatium Weissei*, dem Bodenschlamm und dem darüber

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[1] Siehe hierüber JIMBO, T., Observations on the purple and green bacteria in a sulphur spring at Yumoto, Nikkô. Bot. Mag. Tokyo, **51**, 872, 1937 [auch Ec·'. Rev., **4**, 39, 1938 (Japanisch.)]

[2] Vgl. BRUNCK, O., Zur jodometrischen Bestimmung des Schwefelwasserstoffs. Ztschr. anal. Chem., **45**, 541, 1906.

[3] Chlorobienkolonien vorfinden sich bei Wassertemperaturen von 20-40°C. [Vgl. JIMBO, a. a. O.]

Tabelle 1.

	Ort	H₂S mg/l	Temperatur °C	Anmerkung
	1. Bei der Quelle	30		
	2. ,,	23	29	
	3. Gosse aus Bädern	18 bzw. 20 je nach den Tagen	36 bzw. 37	
	4. ,,	13 an beiden Tagen	27	
Chromatium Weissei[1]	5. Bei der Quelle	9	30	
	6. ,,	9	27	
	7. ,,	3 bzw. 8 je nach den Tagen	24 bzw. 25	
	8. ,,	6	24	Von Chloronium mirabile (= Chloro-chromatium) begleitet
	9. ,,	3	22	
Chromatium vinosum	Bei der Quelle	16	28	Auf faulenden Blättern Kolonien bildend
Chlorobium limicola	1. Bei der Quelle	57		
	2. ,,	49		Schwefelrasenartig
	3. ,,	31		
Schwefelrasen	1. Bei der Quelle	69	63	
	2. ,,	43	63	
Quellendes Thermalwasser	Becken Nr. 1	48	63	
	Becken Nr. 2	47	68	

[1] Es liess sich beobachten, dass die Ausbildung der Kolonien bei Schwefelwasserstoffkonzentrationen von mehr als etwa 10 mg/l stärker geschah.

fliessenden Wasser gefüllten Fläschchen, welches Aufschluss gab über die Beziehung zwischen der Entwicklung dieser Bakterie und der Schwefelwasserstoffkonzentration. Legt man diese Fläschchen ins Licht, so erkennt man öfters innerhalb mehrerer Stunden eine so üppige Vermehrung der Chromatien, dass die Schlammoberfläche von purpurnem Überzug bedeckt wird, und dass überdies die überstehende Flüssigkeit von schwebenden Chromatien mehr oder weniger tingiert wird. Dass die Neubildung von mindestens Schwefelwasserstoff hauptsächlich im Schlamm Anlass zu diesem auffälligen Phänomen gibt, wurde aus analytischen Ergebnissen festgestellt.

Beispielsweise ist das Verhältnis in einem nicht völlig gefüllten Fläschchen von etwa 100 ccm Inhalt hie unten wiedergegeben (Tabelle 2).

<div align="center">Tabelle 2.</div>

mg/l H_2S in der Flüssigkeit[1]					
30. VI.	1. VII.	2. VII.		3. VII.	4. VII.
15^{66} (Beim Füllen)	17^{00}	8^{07}	13^{70}	15^{00}	12^{00}
$13^{2)}$	58^{x}	57^{**}	27^{***}	6	11^{**}

[1] Der Asteriskus zeigt das Sichfärben der Flüssigkeit, deren Färbe-Grad mit seiner Zahl repräsentiert ist. Am 3. Juli hat sich die Flüssigkeit morgens noch gefärbt. Nach dem abermaligen Auftreten der Farbe am Morgen des 4. Juli, das auf nächtlicher Anhäufung von H_2S zu beruhen scheint, verschwand die Farbe nachmittags, und seitdem sah ich sie nicht mehr.

[2] Dieser Wert wurde gleich vor dem Füllen an Ort und Stelle bestimmt.

Falls man das Fläschchen unter vollkommenem Ausschluss der Luft füllt, häuft sich eine ganz enorme Menge von Schwefelwasserstoff bis auf 120 mg/l an. Dabei wurde noch eine kräftige Entwicklung der Chromatien konstatiert.

Wir können uns die Verhältnisse folgendermassen erklären: Sulfat-reduzierende Bakterien,[1] sicherlich in gewissem Ausmass auch eiweiss-zersetzende Bakterien, rufen starke Schwefelwasserstoffentwicklung und demnach ergiebige Chromatienfortpflanzung hervor. Jedoch vermindert sich, besonders infolge des Verbrauchs der für ihr Leben unentbehrlichen organischen Substanzen, die Bakterientätigkeit und dementsprechend auch die Schwefelwasserstoffproduktion nach und nach, und schliesslich treibt die allzu erniedrigte Schwefelwasserstoffkonzentration der Flüssigkeit die schwebenden Chromatien dazu, sich auf der Schlammoberfläche aufzusetzen.

Daraus sind wir berechtigt zu schliessen, dass *Chromatium Weissei* auch bei ganz hohen Schwefelwasserstoffkonzentrationen zu gedeihen imstande ist, und dass die maximale Konzentration (30 mg/l) um die Kolonien an Ort und Stelle das wahre Maximum von Schwefelwasserstoffkonzentration für diese Bakterie durchaus nicht anzeigt. In der Tat konnte ich die Chromatien im gekühlten Thermalwasser aus dem Becken wachsen lassen. Wegen des Fehlens von Schlamm überschritt hierbei die Schwefelwasserstoffkonzentration kaum den ursprünglichen Wert.

[1] Nach rezenter offizieller Prüfung zeigt das Thermalwasser hohen SO_4-Gehalt wie 382 mg/l.

Ferner können wir aussprechen dass der begrenzende Faktor hinsichtlich der örtlichen Verbreitung dieser Bakterie innerhalb der Thermen von Yumoto nicht die Schwefelwasserstoffkonzentration, sondern die Wassertemperatur ist.

Wenn wir auch zunächst nur die Schwefelwasserstoffkonzentration des überstehenden Wassers in Betracht gezogen haben, so ist doch anzunehmen, dass die Schwefelwasserstoffkonzentration immer bedeutend höher im Schlamm, welcher der Hauptsitz der schwefelwasserstoffproduzierenden Bakterien ist, als im Wasser sein muss, und dass das Zusammenkommen der Chromatien an der Schlammoberfläche in den Fläschchen wie auch an den Fundorten[1] durch die chemotaktische Bewegung nach der höheren Konzentration besonders von Schwefelwasserstoff[2] bewirkt wird.

Betrachten wir die Neigung der Chromatien sich in besonders ganz wenig Schlamm enthaltenden Fläschchen um die Schlammteilchen am Boden herum zu versammeln, so können wir diese Annahme mit Recht bestätigen.

Selbstverständlich bleibt die Schwefelwasserstoffkonzentration im Wasser im wesentlichen beständig an den Stellen wo das Thermalwasser fortwährend strömt, sofern sie nicht von Regenfällen gestört wird, und dort können die Chromatien nicht gegen den Strom schweben.

Dagegen können wir die Entwicklung der Chromatien im Wasser bei verschiedenen Schwefelwasserstoffkonzentrationen bei den oben erwähnten Experimenten vergleichen. Aus meinen gesamten Ergebnissen ergibt sich jedoch, dass der Färbe-Grad (i. e. die Menge schwebender Chromatien) überstehenden Wassers in den Fläschchen nicht mit der Schwefelwasserstoffkonzentration gleichlaufend ist. Deutliches Sichfärben wurde nicht einmal bei 3 mg/l H_2S bemerkt, während, wie aus Tabelle 2 ersichtlich ist, die Farbe schon bei 6 mg/l H_2S vergehen konnte. Ähnliche Unregelmässigkeiten kamen auch bei höheren Konzentrationen vor.

Wir können uns dieses komplizierte Verhalten der Chromatien folgendermassen erklären: Prinzipiell neigen die Chromatien dazu sich, mindestens unter den gegebenen Umständen, immer chemotaktisch an der Schlammoberfläche anzusammeln, und sie schweben in der Flüssigkeit lediglich dann, wenn dickerer Chromatienüberzug auf dem Schlamm Schwefelwasserstoffarmut hervorbringt und dadurch ein verkehrtes Gefälle der Schwefelwasserstoffkonzentration stattfinden kann.

[1] Die auf der Schlammoberfläche aufsitzenden Chromatienzellen sind immer im allgemeinen beweglich.

[2] Was Kohlensäure usw. anbelangt, ist zukünftiger Untersuchung zu überlassen.

Nachdem ich erfolglose Versuche zur *Reinzucht von Chromatium Weissei* wiederholt habe, gelang es mir nunmehr die Vermehrung dieser Bakterie in einem Agarmedium folgendermassen zu veranlassen: Man vermischt das Thermalwasser aus dem Becken mit gleichem Volumen verschmolzenen Agars (3:100), impft darin die Chromatien, und dann füllt man damit vollständig eine BÖTTCHERS Kammer. Die BÖTTCHERS Kammer, die für die Kontrolle des Bakterienwachstums unter dem Mikroskope geeignet ist, kann auch durch etwas anderes ersetzt werden. Ich hoffe, dass dieser Erfolg zur Entdeckung irgendeines angemessenen synthetischen Mediums führen wird.

SOME OBSERVATIONS ON *PANDALUS KESSLERI* CZERNIAVSKI

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(With 4 figures in text)

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Pandalus kessleri is an important large, edible species as are other pandaloid shrimps, viz. *Pandalus borealis* and *Pandalus hypsinotus* from North Japan.

Both *Pandalus borealis*[1] and *Pandalus hypsinotus*[2] inhabit the deep seas and are distributed over a wide area, while *Pandalus kessleri* occurs only in shallow waters and its area of distribution is narrow. The latter species may be looked on as a special product of Saghalien and Hokkaidô, even though its southern limit is Iwate Prefecture. In Saghalien, it is the only commercial shrimp that is canned.

Both of the present writers here wish to express their sincere thanks to Dr. MARY J. RATHBUN, Dr. Y. YOKOYA and Prof. K. SATÔ for their kind information, to Dr. WALDO L. SCHMITT for his kind help, owing to which the writers were able to compare their species with *Pandalus platyceros*, and to the late Mr. J. HORI, Mr. K. KAWADA, Mr. G. YAMADA, Mr. Y. YAMAYA, Mr. S. HATTORI, Mr. K. WADA and Mr. G. TOBA for their kindness in collecting the specimens.

THE SPECIFIC NAME

In 1902, F. DOFLEIN made the first report on the shrimp from Muroran with a beautiful illustration printed in three colours, saying that " Abge-

[1] Distribution: Circum-Polar, North Europe, North America, Okhotsk Sea and North Japan. In Japan, abundant in the neighbourhood of the Noto Peninsula, about 100 fms. deep. Depth of habitat: 29.5-495 fms.

[2] Distribution: North Pacific Ocean from Bering Sea southwards to Fuca Strait and Japan. In Japan, abundant in Volcano Bay, Hokkaidô; Hirasawa, Akita Prefecture; Toyama Bay and the east coast of Tyôsen. Depth of habitat: 36-495 m.

sehen von der Färbung welche die früheren Autoren nicht erwähnen, stimmen meine erwachsenen Exemplare vollkommen mit der Beschreibung des *P. platyceros* von BRANDT, meine jungen Stadien mit *P. gracilis* nach STIMPSON überein." In the same year, MARY J. RATHBUN reported the existence of the new species, *Pandalus latirostis*, from the same locality. In 1907, a species from Aniwa Bay, Saghalien, was identified by V. BRAJNIKOV[1] with *Pandalus kessleri* CZERNIAVSKI (1878, Olga Bay on the Soviet coast of the Japan Sea). In 1914, H. BALSS gave the name, *Pandalus platyceros*, to the species from Muroran, Nemuro and Vladivostock, and stated that both *P. latirostris* and *P. kessleri* were corresponding designations for it. "*P. kessleri*," says DE MAN in 1920, "seems to be identical with the species, *P. latirostris* RATHBUN.....*P. platyceros* J. F. BRANDT has, however, erroneously been identified by BALSS (Ost. Decap. II. 1914, P. 28) with *P. kessleri* CZERN.=*P. latirostris* RATHB., for it differs at first sight by lower margin of the rostrum being only armed with five teeth and by the antennal scale being distinctly shorter than the carapace." In 1924, H. BALSS corrected his opinion and says that "Die von DOFLEIN und mir als *Pandalus platyceros* BRANDT bestimmten Formen gehören, wie DE MAN (1920, p. 104) bemerkt, in wirklichkeit zu *P. kessleri* CZERNIAVSKI (=*P. latirostris* RATHBUN). *Pandalus platyceros* BRANDT ist also aus der Fauna Japans zu streichen." Thus, DOFLEIN's beautiful illustration is not that of *P. platyceros* but of *P. kessleri*. Among authorities, however, there are some who use *Pandalus latirostris* RATHBUN for the species even at present, but MARY J. RATHBUN says in a letter to URITA on December 18, 1934, "I think *latirostris* is a synonym of *kessleri*. The latter species probably was unkown to me at the time of naming." Finally, the description of RATHBUN (1902) and of BRAJNIKOV (1907) agrees most with the characteristics of the present writers' species. The following specific name has, therefore, been decided on for the species.

<div align="center">

Pandalus kessleri CZERNIAVSKI

Nom. Jap. — Hokkai-Ebi

</div>

Pandalus kessleri CZERNIAVSKI, 1878, p. 23; BRAJNIKOV, 1907, p. 106, text-fig. 12 a–b, Pl. 2, figs. 7, 8; DE MAN, 1920, p. 104; BALSS, 1924, p. 24.

Pandalus latirostris, RATHBUN, 1902, p. 46, text-figs. 20, 21; YOKOYA, 1930, p. 538.

Pandalus platyceros, DOFLEIN, 1902, p. 635 (part), Pl. 1, figs. 1, 2; BALSS, 1914, p. 28 (part).

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[1] This is spelt 'BRASHNIKOW' by Dr. BALSS and 'BRAŽNIKOV' by Dr. RATHBUN in Latin style. But, according to Prof. Dr. K. OKADA's account, the person himself and his family use 'BRAJNIKOV' and the present writers wish to follow them.

THE EXTERNAL FEATURES

1) The Measurements :

TABLE 1

The measurements of 45 males (130 157 mm.) and of 11 females (132–160 mm. in total length) of *Pandalus kessleri*.

	Range		Mode		Mean		S. D.		C. V.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Carapace	100	100	100	100	100	100				
Rostrum	120~148	109~141	133	123	133.29 ±0.63	126.19 ±0.80	6.30 ±0.15	7.64 ±0.57	4.73 ±0.31	6 06 ±0.45
Scale	100~122	94~119	107	108	110.14 ±0.59	107.83 ±0.51	5.86 ±0.42	4.81 ±0.36	5.32 ±0.38	4 46 ±0.33
3rd pereiopod	115~174	150~175	162	159	159.94 ±0.60	160.29 ±0.61	5.99 ±0.43	5.71 ±0.43	3.75 ±0.27	3.56 ±0.27
Abdomen	233~268	229~269	252	252	249.07 ±0.81	246.78 ±1.09	8.01 ±0.57	10.32 ±0.77	3.21 ±0.23	4.18 ±0.31
6th abdominal somite	40~48	41~47	45	12	44.82 ±0.17	43.41 ±0.18	1.71 ±0.12	1.67 ±0.12	3.82 ±0.27	3.85 ±0.29
Telson	63~76	63~76	70	68	71.31 ±0.30	69.81 ±0.30	3.02 ±0.22	2.85 ±0.22	4.24 ±0.30	4.09 ±0.30

URITA has once measured a female of 181 mm. and a male of 160 mm. in total length, as the largest specimens of this species. The male appears, as a rule, to be a little smaller than the female.

2) The Branchial Formula :

	h	i	k	l	m	n	o
Pleurobranchiae	0	0	1	1	1	1	1
Arthrobranchiae	0	1	1	1	1	1	0
Podobranchiae	1 rud.	0	0	0	0	0	0
Mastigobranchiae	1	1	1	1	1	1	0

3) The Colour of the Body. When alive, the shrimp is not yellow in colour nor so beautiful as shown in DOFLEIN's picture. When dipped in formalin, however, its colour changes approximately to that in the picture.

The ground colour of the body is brown, sometimes assuming a greenish brown or dark brown, probably owing to the colouration of the sea-weeds, among which the shrimp lives.

It is ornamented with longitudinal streaks, some dark some whitish, the dark ones alternating with the whitish ones. The whitish one looks

whitish, because it contains about four slender, somewhat wavy, longitudinal, whitish lines bordered by the brown colour of the ground.

a

b

Fig. 1. *Pandalus kessleri* CZERNIAVSKI. *a* ♂, 79 mm. *b* ♀, 172 mm. in total length.

On the dorsal surface of the body, there are three whitish streaks and between them two dark ones. The median whitish streak extends backwards to the fifth abdominal somite, but is narrow and very indistinct. The two whitish streaks on both sides of the median one are distinctly marked. Each of them begins on either side of the rostrum, and reaches as far as the telson.

On the lateral side of the body, there are three whitish streaks alternating with three or four dark ones. The first or uppermost dark streak begins at the orbit and ends at the telson. The second dark streak extends from the antennal spine to the posterior spine of the pleuron of the sixth abdominal somite. The third dark streak runs from the pterygostomian spine to the fourth abdominal somite. The fourth or last dark streak is obscure, and sometimes unnoticeable. The whitish streaks between

the dark ones are distinct, with the exception of the last one which is obscure.

The immature specimen has one dark streak on the dorsal surface, and two dark ones on either side of the body. All the three are especially distinct.

4) The Distinctions between *P. kessleri* CZERNIAVSKI and *P. platyceros* BRANDT[1]. *P. kessleri* differs in the following points from *P. platyceros*, which has erroneously been identified with the former species.

Fig. 2. *Pandalus platyceros* BRANDT. ♂, 163 mm. in total length.

In *P. kessleri*, the rostrum is strongly formed and usually of the same length as the antennal scale or a little longer. The rostral formula is $4-5+\frac{8-17}{7-14}+2$. The lateral ridge is prominent, extending to the bifid tip. In *P. platyceros*, the rostrum is rather slender, long and curved. It exceeds the antennal scale by two-fifths of its length. The rostral formula is $4-5+\frac{9-13}{7-8}+2$. The lateral ridge disappears at about the middle of the rostrum. A solitary spine is situated not far behind the acute tip.

In *P. kessleri*, the basal spine of the antennule is round at the end, and extends to the middle of the first segment of the antennular peduncle. The flagellum is short and its greater part is hidden between the rostrum and the antennal scale. In *P. platyceros*, the end of the basal spine is

[1] It is regrettable that the present writers could not obtain a Japanese specimen of *P. platyceros*. Their description, which was here used, was based on a specimen Albatross Stat. 3188, 36° 31′ N. 121° 59′ W., 162 fms., Apr. 3, 1890, indentified by MARY J. RATHBUN, which was sent to URITA by the National Museum, U. S. A., through the good offices of Dr. WALDO L. SCHMITT.

pointed, and reaches almost to the anterior margin of the first segment
of the peduncle. The flagellum is very long, and projects far beyond
the antennal scale.

In *P. kessleri*, the antennal scale is longer than the carapace. The
end of the blade is very oblique outwardly, and projects considerably
beyond the outer spine. In *P. platyceros*, the antennal scale is shorter
than the carapace. The extremity of the blade is subtruncate, and is
slightly exceeded by the spine in length.

In *P. kessleri*, the outer maxilliped reaches to about the half of the
antennal scale, while, in *P. platyceros*, it reaches the end of that scale.

The measurement of *P. platyceros* is shown in Table 2.

TABLE 2

The measurement of *Pandalus platyceros* BRANDT

($♂$, 163 mm. in total length).

	Carapace	Rostrum	Scale	3rd pereiopod	Abdomen	6th abdominal somite	Telson
Observed value in mm.	31.0	57.0	30.5	66.0	75.0	13.0	21.5
Value taking carapace as 100	100	184	98	213	242	42	69

P. kessleri usually occurs at a depth of 0.5-4 fms., but *P. platyceros*
is found mostly at a depth of 100-266 fms. and only a few instances are
known at the depth of 20-60 fms.

The distributional area of *P. platyceros* is wide. In North America,
it is distributed from Unalaska in the Aleusian Is. to San Diego (about
Lat. 33° N.). In Asia, no record of any locality in which it occurs is
known, so far as the present writers are aware, except that, according
to Dr. YOKOYA, only one male specimen was caught in the south of
Sado I. (Sôyô Stat. 591, Aug. 3, 1930), 311 m. deep, though some
authorities are of opinion that the species occurs on the east coast of
Hokkaidô, at Hakodate, etc. The distributional area of *P. kessleri* is
narrow being limited to North Japan.

THE ROSTRAL FORMULA AND ITS VARIATION

The teeth formula of the rostrum or rostral formula of *P. kessleri*
is $4-5 + \dfrac{8-17}{7-11} + 2$ or $\dfrac{12-22}{7-14} + 2$. It is, therefore, widely variable in

comparison with those of the pandaloid shrimps found in North Japan, as seen in Table 3.

TABLE 3

The rostral formulae of pandaloid shrimps of North Japan.

Species	Rostral formula	Ranks of variation	
		Upper marginal teeth	Lower marginal teeth
P. nipponensis	$\frac{12-16}{11}+2$	5	1
P. platyceros	$\frac{14-17}{7-8}+2$	4	2
P. hypsinotus meridionalis	$\frac{10-15}{6-7}+2-3$	6	2
P. goniurus	$\frac{7-10}{5-8}+2$	4	4
P. borealis	$\frac{11-16}{6-9}+2$	6	4
P. hypsinotus	$\frac{17-22}{7-10}+3$	6	4
P. kessleri	$\frac{12-22}{7-14}+2$	11	8

For the purpose of solving the question whether the irregularity in number of the rostral teeth[1] depends upon the local variation or not, URITA collected specimens from five places in Saghalien, viz. Maoka on the west coast, Lake Tôbuti and Ôtomari on the coast of Aniwa Bay, Tirie and Lake Ondô at Tonnai Village on the east coast, Nemuro, Hokkaidô, and Ôma, Aomori Prefecture, and. counted the number of rostral teeth for comparison.

The result found by counting the movable teeth on the upper margin of the rostrum shows, as indicated in Table 4 a, that, in Saghalien the normal number is invariably 16, Ôtomari (17), Nemuro (17) and Ôma (18) being exceptions.

The number counted of the immovable teeth on the lower margin is shown in Table 4 b. Saghalien, Nemuro and Ôma show invariably the mode 11, Maoka (10) being an exception.

In order to make clear the difference in the number of the rostral teeth between two selected localities, the present writers ascertained the

[1] The rostral teeth include all the movable teeth on the carapace, but not the bifid immovable teeth at the tip of the rostrum.

TABLE 4

The number of the rostral teeth of *Pandalus kessleri*.

a) The upper margin.

Locality		Range	Mode	Mode coefficient	Mean	S. D	C. V	Number of specimens
Saghalien	Maoka	13~21	16	30.06%	16.28±0.09	1.39±0.07	8.55±0.41	219
	Lake Tôbuti	13~21	16	25.70	16.48±0.10	1.62±0.07	9.88±0.43	276
	Ôtomari	13~22	17	25.68	16.76±0.10	1.61±0.07	9.76±0.43	257
	Lake Ondô	12~22	16	24.30	16.64±0.09	1.56±0.06	9.35±0.37	321
	Tirie	13~21	16	31.90	16.41±0.09	1.34±0.07	8.12±0.40	210
All Saghalien		12~22	16	26.02	16.53±0.04	1.53±0.03	9.25±0.51	1283
Nemuro		13~22	17	24.26	17.30±0.11	1.64±0.08	9.50±0.43	239
Ôma		13~19	18	34.29	16.60±0.18	1.56±0.13	9.00±0.00	70

b) The lower margin.

Locality		Range	Mode	Mode coefficient	Mean	S. D	C. V	Number of specimens
Saghalien	Maoka	7~14	10	32.40%	10.59±0.08	1.12±0.06	11.30±0.55	219
	Lake Tôbuti	7~14	11	34.79	10.69±0.07	1.14±0.05	10.70±0.46	276
	Ôtomari	7~14	11	39.65	10.77±0.10	1.64±0.07	15.20±0.67	257
	Lake Ondô	8~14	11	36.75	10.69±0.06	1.02±0.04	9.57±0.38	321
	Tirie	8~14	11	32.38	10.47±0.08	1.17±0.06	11.20±0.56	210
All Saghalien		7~14	11	35.07	10.65±0.03	1.11±0.02	10.40±0.66	1283
Nemuro		8~14	11	32.63	11.22±0.08	1.20±0.05	10.70±0.49	239
Ôma		8~13	11	28.57	10.54±0.15	1.22±0.11	12.00±0.40	70

standard error of the difference between the means (S. D.) of the localities, and, making it the unit of measurement, measured the difference between the means of the localities, the resulting value, " t ", being thus obtained. Here, an example is given as to the teeth on the lower margin of the rostrum of the specimens from Ôtomari (Ô) and of those from Tôbuti (T).

The mean of Ô = 10.767, and that of T = 10.689.

The S. D. of Ô = 1.643, and that of T = 1.142.

The number of specimens of Ô = 257, and that of T = 276.

$$\text{S. D.} = \sqrt{\dfrac{\dfrac{1.643^2 \times 257 + 1.142^2 \times 276}{(257-1)+(276-1)}}{\dfrac{257 \times 276}{257 + 276}}} = 0.122$$

The difference between the means of Ô and T is

$$10.767 - 10.685 = 0.082$$

$$\therefore \quad \text{``t''} = \frac{0.082}{0.122} = 0.673$$

Generally, when the multiple of standard error does not exceed 2.576 (in the case that the number of specimens is large), we can interpret that 99 percent out of 100 of specimens are picked up from the same population. Since 0.673 is far less than 2.576, it might said that the difference of the means between Ô and T is not significant, i. e. any local variation between Ô and T can not be found.

TABLE 5

"t"s of both the upper and lower marginal teeth of the rostrum of the specimens from two selected localities.

Lower marginal teeth		Maoka	Lake Tôbuti	Ôtomari	Lake Ondô	Tirie	Upper marginal teeth
	Maoka	——	1.44	3.38	2.76	0.99	
	Lake Tôbuti	0.97	——	1.98	1.23	0.51	
	Ôtomari	1.38	0.67	——	0.90	2.48	
	Lake Ondô	1.07	0.00	0.72	——	1.75	
	Tirie	1.08	2.10	2.30	2.28	——	

Lower marginal teeth		Saghalien	Nemuro	Ôma	Upper marginal teeth
	Saghalien	——	7.07	0.37	
	Nemuro	7.17	——	3.87	
	Ôma	0.80	4.14	——	

In Saghalien, as shown in Table 5, the "t"s of the upper marginal teeth are 0.51~3.38, and those of the lower marginal 0.00~2.30. Therefore, we may conclude that no, or only a little, difference in this respect exists between the localities in Saghalien. The value of "t" between Saghalien and Ôma being 0.37 in the upper marginal teeth and 0.80 in the lower marginal teeth, there is also only an insignificant difference between the two localities in regard to these values. The value of "t" between Saghalien and Nemuro is 7.07 in the upper and 7.17 in the lower marginal teeth, and that between Nemuro and Ôma is 3.87 in the upper and 4.14 in the lower. Therefore the differences between those localities in regard to these values should be considered significant.

The upper and lower marginal teeth of *P. kessleri* have respectively 11 and 8 ranks of variation, but the differences in this respect between

the localities are not significant, though two notable differences are found between the specimens from Saghalien and those from Nemuro, and also between those from Nemuro and those from Ôma.

As regards the rostal formula, the 'Variationsbreite' is very wide, and, theoretically, it ought to be $\frac{12-22}{7-14}$, i. e. $11 \times 8 = 88$, but it is, in reality, only 43, i. e. about half of 88, even in the case of specimens from Lake Ondô, which show the widest 'Variationsbreite' in Saghalien.

In order to indicate the frequency of the rostral formula, the best method is to make a correlation table between the number of teeth on the upper and the number of teeth on the lower margin of the rostrum, as in Table 6 for instance.

TABLE 6

The correlation table between the upper and lower marginal
teeth of the rostrum of the specimens from
the localities in Saghalien.

U \ L	12	13	14	15	16	17	18	19	20	21	22	Σ	Max
7		1		1			1	1				4	16.25
8		1	2	6	5	8	4	1				27	16.22
9	1	4	15	22	40	37	12	10	3			144	16.24
10		4	36	74	109	89	48	23	9	1		393	16.35
11	1	6	24	73	123	110	67	30	12	2	1	449	16.60
12		4	6	34	44	50	39	21	7	3	1	209	16.90
13				4	12	18	13	2		1		50	17.02
14					1	2	2	1	1			7	17.90
Σ	2	20	83	214	334	314	186	89	32	7	2	1283	
May	10.00	10.25	10.21	10.54	10.60	10.70	10.91	10.80	10.84	11.60	11.50		

$$\text{Max} = 16.53 \pm 0.04 \qquad \text{May} = 10.65 \pm 0.03$$
$$\delta x = 1.53 \pm 0.03 \qquad \delta y = 1.11 \pm 0.02$$
$$\gamma = 0.17 \pm 0.03$$

U   Number of upper marginal teeth   L   Number of lower marginal teeth

As shown in Table 7, the correlation tables of the localities show that there are three kinds of the mode in Saghalien, viz. $\frac{16}{10}$ at Maoka on the west coast, $\frac{16}{11}$ at Lake Ondô and Tirie on the east coast and $\frac{17}{11}$ at Lake Tôbuti and Ôtomari on the coast of Aniwa Bay, and that for Saghalien, as a whole, the mode is $\frac{16}{11}$, at Nemuro $\frac{17}{11}$, and at Ôma $\frac{18}{11}$.

TABLE 7

The modes of the rostral formula of specimens collected
at Saghalien, Nemuro and Ôma.

	Locality	Mode	Mode coefficient
	Maoka	$\dfrac{16}{10}$	11.40%
Saghalien	Lake Tôbuti	$\dfrac{17}{11}$	10.15
	Ôtomari	$\dfrac{17}{11}$	9.33
	Lake Ondô	$\dfrac{16}{11}$	9.66
	Tirie	$\dfrac{16}{11}$	11.43
	All Saghalien	$\dfrac{16}{11}$	9.58
	Nemuro	$\dfrac{17}{11}$	8.37
	Ôma	$\dfrac{18}{11}$	8.57

As regards the density of frequency of the rostral formula, in Saghalien the mode coefficient is from 9.33 at Ôtomari to 11.43 at Tirie, and in Saghalien, as a whole, it is 9.58, at Nemuro 8.37 and at Ôma 8.57. Therefore, the frequency of the rostral formula is widely distributed and its individual variation is very significant.

The result of the computation of the correlation coefficients 'γ' between the numbers of the upper and lower marginal teeth is 0.17 ± 0.03 for Saghalien, as a whole. This shows that the correlation between them is scant.

In conclusion, the rostral formulae show a remarkable individual variation and a slight local variation.

THE GROWTH RELATION

Pandalus kessleri, when young, has a long rostrum and a long carapace in comparison with its whole body length. But, when old, both rostrum and carapace appear to be comparatively short. In order to determine the growth relation between them URITA measured the total length of the body (L) and the length of the cephalo-thorax (A), of the abdomen (B), of the rostrum (C) and of the carapace (D) of the specimens collected from Lake Ondô on October 7, 1937, using compasses and callipers.

TABLE 8

Measurements of *Pandalus kessleri* (♀) collected at Lake Ondô.

Total length in mm.	Number of specimens	Average length			
		Cephalo-thorax	Abdomen	Rostrum	Carapace
55	1	28.0	27.0	18.0	10.0
56	2	29.0	27.0	18.5	10.5
57	1	29.0	28.0	18.0	11.0
58	3	30.0	28.0	18.0	11.0
59	2	30.6	28.3	19.6	11.0
60	1	31.5	28.5	19.5	11.5
61	1	32.0	29.0	20.0	12.0
62	2	31.8	30.3	20.0	11.8
63	2	33.0	30.0	20.8	12.3
64	1	32.5	31.5	21.0	11.5
65	3	34.0	31.0	21.7	12.3
66	1	34.0	32.0	21.0	13.0
67	3	34.7	32.3	21.5	13.2
68	4	34.9	33.1	22.0	12.9
69	5	36.0	33.2	23.0	13.0
70	12	36.2	34.0	22.8	13.5
71	9	36.7	34.5	23.6	13.1
72	7	37.1	35.0	23.1	13.9
73	3	38.0	35.0	24.0	14.0
74	3	38.3	35.7	24.3	14.0
75	7	38.4	36.6	23.9	14.5
76	6	39.0	37.1	24.5	14.2
77	2	39.0	38.0	24.5	14.5
78	8	40.1	37.9	25.1	14.9
79	4	40.4	38.6	25.6	14.6
80	4	41.6	38.9	26.0	15.1
81	6	41.5	39.6	26.0	15.4
82	6	42.2	40.0	26.3	15.6
83	6	42.3	40.8	26.5	15.6
84	4	43.1	40.9	27.2	15.9
85	8	43.3	42.0	27.0	16.4
86	4	43.8	42.2	27.2	16.5
87	5	44.1	42.9	27.3	16.9
88	8	44.7	42.8	28.0	16.6
89	3	45.0	44.0	28.3	16.8
90	9	45.6	44.4	28.4	17.1
91	3	46.7	44.3	29.8	16.8
92	9	46.9	45.1	29.3	17.6
93	3	47.7	45.3	29.3	18.3
94	3	47.5	46.5	29.3	18.2
95	2	47.8	47.3	28.8	19.0
96	5	48.8	47.2	30.5	18.3
97	4	49.5	47.2	30.9	18.6
98	5	49.5	48.6	30.6	18.8
99	7	50.0	49.2	30.9	19.0
100	6	50.3	49.7	30.9	19.4
101	3	51.0	50.0	31.0	20.0
102	1	51.0	51.0	32.0	19.0
103	4	52.0	51.0	32.1	19.6
104	1	51.0	53.0	31.0	20.0
105	3	52.2	52.8	31.7	20.5
106	2	53.3	52.7	32.3	21.0
107	1	53.0	54.0	31.0	22.0

Total length in mm.	Number of specimens	Average length			
		Cephalo-thorax	Abdomen	Rostrum	Carapace
108	2	53.5	54.5	32.0	21.5
109	2	53.5	55.5	32.5	21.0
110	11	54.1	55.7	32.9	21.4
111	2	55.3	55.8	33.0	22.3
112	5	55.2	56.8	33.0	22.5
113	1	56.0	57.0	33.0	24.0
114	4	56.3	57.8	34.5	21.8
115	5	56.8	58.2	34.6	22.2
116	5	57.2	58.8	34.7	22.5
117	4	57.5	59.5	34.5	23.0
118	4	58.8	59.0	35.3	23.5
119	6	59.1	59.2	35.4	23.8
120	5	59.2	60.6	35.8	23.6
121	3	59.0	62.0	35.2	23.8
122	3	60.0	62.0	35.5	24.5
123	5	61.2	62.1	36.5	24.7
124	4	60.8	63.3	36.0	24.8
125	2	61.3	63.8	36.8	24.5
126	1	62.5	63.5	38.5	24.0
127	3	61.8	65.2	36.7	25.2
128	6	62.0	66.0	36.5	25.5
129	2	64.0	65.0	36.0	26.5
130	10	63.4	66.7	37.5	26.3
131	3	63.7	67.3	37.0	26.7
132	1	64.0	68.0	37.0	27.0
133	3	65.3	67.7	38.3	27.0
134	2	65.0	69.0	37.5	27.5
135	1	66.5	68.5	39.0	27.5
136	4	65.6	70.6	37.0	28.4
137	2	66.5	70.3	39.0	27.5
138	2	66.3	71.8	37.5	28.8
139	3	67.5	71.3	39.7	27.8
140	4	68.4	71.6	38.5	29.9
141	3	68.3	72.7	39.3	29.0
142	1	68.5	73.5	39.0	29.5
143	2	69.5	73.0	39.0	30.5
144	4	69.8	74.3	39.6	30.1
145	4	69.8	75.3	39.5	30.3
146	3	69.7	76.3	39.7	30.0
147	2	70.0	77.0	40.0	30.0
148	3	71.7	76.3	40.7	31.0
149	1	72.0	77.0	40.0	32.0
150	2	73.0	77.5	41.5	31.0
157	1	74.0	83.0	43.0	31.0

Of 397, the total number of the specimens then examined, only 41 were female. They had the stylamblys on the endopodite of the second abdominal appendage, but not the masculine appendix. All the females were of large size with a body length of over 132 mm. The remaining specimens had invariably both the stylamblys and the masculine appendix, which are the male characteristics of the species. Accordingly, in the determination of the growth relation the male specimens were tested exclusively.

The growth relation between the total body length and the length of the cephalo-thorax or abdomen satisfies the following equations:

$$A = 0.799 \, L^{0.9} \qquad B = 0.330 \, L^{1.00}$$
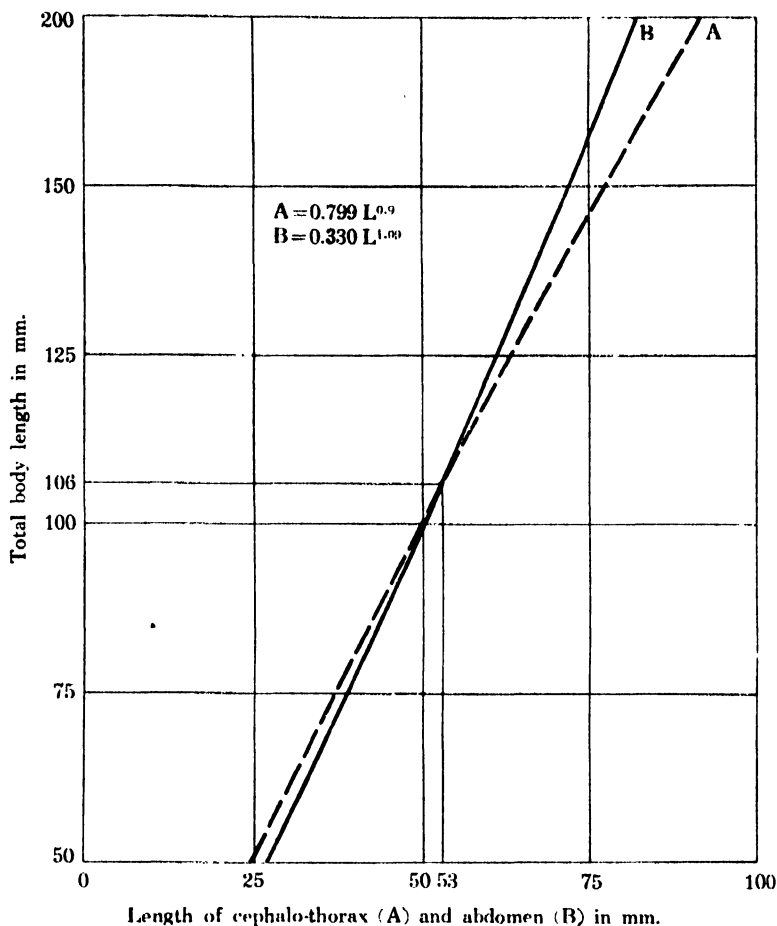
Fig. 3. The growth curves of the male of *Pandalus kessleri*.

As shown in Fig. 3, these two growth curves intersect each other at the point of the total length 106 mm. This shows that, when the total body length is above 106 mm., i. e. in the older stage, the cephalo-thorax is shorter than the abdomen and, when it is below 106 mm., i. e. in the younger stage, the reverse is the case.

The growth relation between the total body length and the length of

the rostrum or the carapace satisfies the following equations:
$$C = 0.800 \, L^{0.79} \qquad D = 0.123 \, L^{1.10}$$

TABLE 9

Total length in mm.	$\dfrac{R}{R \,\&\, C} \times 100$	$\dfrac{C}{R \,\&\, C} \times 100$
55 - 65	63.2	36.8
66 75	62.9	37.1
76- 85	62.8	37.2
86- 95	62.2	37.8
96-105	61.3	38.7
106 115	59.9	40.1
116-125	59.8	40.2
126-135	58.7	41.3
136-145	57.1	42.9
146-150	56.7	43.2

R — rostrum,   C — carapace,   R & C — cephalo-thorax.

Actually, the rostrum is always longer than the carapace. But, as shown in Table 9, the percentage ratio of the rostral length to the cephalo-thoracic length decreases, and that of the carapace length to the cephalo-thoracic length increases with the increase of the body length.

Therefore, the fact, that the cephalo-thorax grows shorter relatively to the growth of the body length, chiefly depends upon the retardative growth of the rostrum.

THE HABIT AND THE HABITAT

Pandalus kessleri occurs abundantly in cold, shallow waters, 0.5–4 fms. deep, where it is chiefly grown among such sea-weeds as *Zostera pacifica* S. WATSON (Nom. Jap. Amamo) and *Phyllospadix scouleri* HOOK (Nom. Jap. Sugamo), sometimes with kinds of *Laminaria*, of *Sargussum*, of *Ulva*, etc.

In Saghalien, the fishing season of this shrimp is ordinarily from May or June to October, but sometimes it is caught in waters, the surface of which is frozen over.

Outside Saghalien, it is known that this species occurs on the coast of Hokkaidô[1], of the northern part of Tôhoku District of Honsyû and

[1] According to a letter from Mr. K. KOBA, *Pandalus kessleri* has not been found on the northern Kurile Is.

on the northern part of the east coast of Tyôsen.

In Saghalien, *Pandalus kessleri* is especially abundant in the following places :

Aniwa Bay :   Lake Tôbuti (1)[1] to 2 fms. deep ; Nobori (2), Notoro Village.

Western coast :   Esutoru (3); Usiro (4), 1–2 fms. deep.

Eastern coast :   Lake Ondô (5) to 1.5 fms. ; the coast of Tonnai Village ; Noto (6), Tirie Village, 1–4 m. deep.

In Hokkaidô, the species is abundant on the coast of the Pacific Ocean and of the Okhotsk Sea, but on the Japan Sea side it is not known whether or not the species occurs to the south of Rebun Is.   The following places are the chief habitats of the species (The Hokkaidô Fisheries Experimental Station).

Kitami Province :   Abasiri (7) and its neighbourhood, 1–1.5 m. deep,
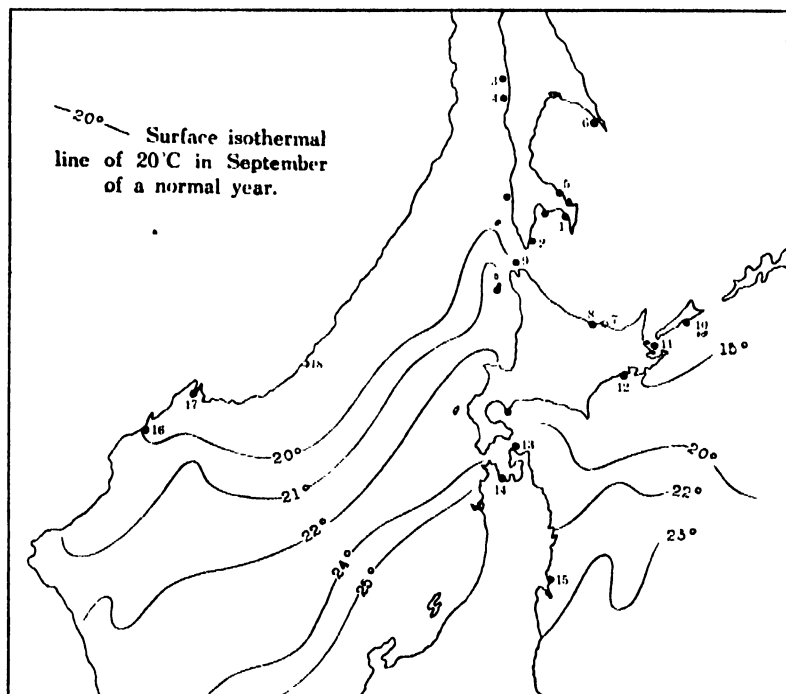
Fig. 4.   The map of North Japan and adjacent territories, showing localities of *Pandalus kessleri*.   17 Vladivostok, 18 Olga Bay, and the remainders in text.

[1] The number in parentheses corresponds to the number of the place indicated in Fig. 4.

abundantly grown in *Phyllospadix scouleri* on the bottom of sandy mud; Lake Nôtori; Lake Saroma (8); Sôya (9) and its neighbourhood, 0.5–8 m. deep.

Nemuro Province: Kunasiri Is. (10); Nokke (11) and its neighbourhood.

Kusiro Province: Lake Akkesi (12), 1–1.5 m. deep, grown among sea-weeds on the bottom of sandy mud.

In Honsyû[1], on the Pacific coast, the following places are known to be the habitat of *Pandalus kessleri*.

Aomori Prefecture: Ôma (13) (YOKOYA), 1–9 m. deep, growing with *Phyllospadix scouleri* and *Sargassum* (Y. YAMAYA coll.); Harabetu, Aomori (14) (K. WADA coll.).

Iwate and Miyagi[2] Prefecture: Otomo-ura, Hirota Bay (15), 2–3 m. deep, grown with *Zostera pacifica* (G. TOBA coll.).

On the coast of Japan Sea, according to Dr. Y. YOKOYA, he obtained this species in winter from the market at Nô, Niigata Prefecture.

In Tyôsen (The Fisheries Experimental Station of Tyôsen Goverment-general), *Pandalus kessleri* is found from Rosanpaku to Yusin, Funei-gun, Kankyô-hokudô (16), about 42° N., in the sea-weed zone.

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[1] Tôkyô (RATHBUN). If it were obtained in the market, it probably came from the northern sea.

[2] According to Dr. Y. YOKOYA, *Pandalus kessleri* has not been found in the collection at Onagawa Bay, Miyagi Prefecture.

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ÜBER DIE BEFRUCHTUNG UND DIE IHR FOLGENDE ERSTE KERNTEILUNG BEI SARGASSUM[1]

VON

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(Mit Tafel X und 2 Textfiguren)

'Eingegangen am 14. Oktober 1938)

In Japan ist die Gattung *Sargassum* schon mehrmals von mehreren Forschern zum Gegenstande der zytologischen Untersuchung gemacht worden. In 1926 veröffentlichten TAHARA und SHIMOTOMAI eine kurze Mitteilung über die Mitosen bei *Sargassum*. Sie konnten in der ersten Metaphase der Reduktionsteilung des Oogoniums von *Sargassum enerve* 32 Gemini erkennen. Später teilte KUNIEDA (1928) über die Kernteilung und Befruchtung von *Sargassum Horneri* etwas umfassendere Resultate mit. Aber seine Ergebnisse stimmen mit denen der vorhergehenden Forscher in einigen Punkten nicht überein. KUNIEDA hat bei der Oo- und Spermatogenese bei *Sargassum Horneri* 16 haploide Chromosomen gezählt. Sein Ergebnis wurde aber durch OKABES nachherige Untersuchung (1929) über die Oogenese bei dieser Art und durch meinige (1933) über die Mitosen im Antheridium von *Sargassum confusum* sehr zweifelhaft. Es ist merkwürdig, dass nach den bisher durchgeführten Untersuchungen verschiedener Forscher, vom Ergebnis KUNIDEDAS abgesehen, alle Arten unter den Fucaceen, z. B. *Fucus vesciculosus* (YAMANO-UCHI 1909), *Fucus evanescens* (INOH 1935), *Pelvetia Wrightii* (INOH 1935), *Cystophyllum sisymbrioides* (SHIMOTOMAI 1928) und *Coccophora Langsdorfii* (TAHARA 1929) sämtlich 32 haploide Chromosomen besitzen.

Dann kommt noch eine andere Frage. Betreffs der Stelle, wo die erste Teilung des befruchteten Eikerns stattfindet, behaupten TAHARA und SHIMOTOMAI (1926), dass bei *Sargassum tortile* die erste Teilung des befruchteten Eikerns nicht im Zentrum, sondern in der Peripherie der Eizelle erfolgt. Diese bemerkenswerte Tatsache haben sie durch eine schematische Abbildung verständlich gemacht. KUNIEDA hat aber ein anderes Resultat mitgeteilt; nach ihm vollziehen sich die Befruchtung und die ihr folgende erste Kernteilung bei *Sargassum Horneri* nicht in

der Peripherie der Eizelle, sondern im Zentrum. Meine vorliegende Arbeit ist hauptsächlich unternommen worden, um diese Frage klar zu machen. Als Material wählte ich auch *Sargassum tortile*, welche in der Umgebung der Biologischen Station zu Asamushi üppig wächst.

Um die Beobachtung in demselben Individuum in jeder Zeit anstellen zu können, verknüpfte ich ein männliche und eine weibliche Pflanze mit einem Stein an einem Pfahl des Hafendamms der Station. Morgen am 13 Juni dieses Jahres begannen die Karyokinesen in den Antheridien der oben genannten Pflanze. Die gleichen Stadien in Oogonien wurden erst im Nachmittag desselben Tages bemerkt. Und kurz vor dem Tagesanbruch des nächsten Tages fand ich die frühere Entleerung der achtkernigen Eier. Gegen 7–8 Uhr erreichte ihre Entleerung den Höhepunkt. Um diese Zeit konnte ich unter dem Mikroskop zahlreiche, um die Eier schwimmende Spermatozoiden erkennen.

Die Fixierungen erfolgten in verschiedenen Zeiträumen. Als Fixierungsmittel benutzte ich dieselbe Lösung, welche ich bei meiner Untersuchung (1932) der Befruchtung von *Coccophora* mit gutem Erfolg gebraucht hatte. Das Material blieb in dieser Lösung gewöhnlich 3–4 Stunden. Die etwa 10 μ dick geschnittenen Mikrotomschnitte wurden mit HEIDENHAINS Eisenalaunhämatoxylin gefärbt.

Die ausgetretenen Eier sind rundlich bis ellipsoidisch und enthalten zahlreiche Chromatophoren um die acht Kerne. Nach dem Eindringen der Spermatozoiden vergrössen sich die Eier allmählich und beginnen einige von den acht Kernen zu grunde zu gehen. Wie aus Taf. X, Fig. 1 hervorgeht, nähert sich der Spermakern einem Eikern in der Peripherie der Eizelle und verschmilzt dort mit ihm (Fig. 2–4). In diesem Stadium bemerkt man im Kern meistens zwei Kernkörperchen, von denen wahrscheinlich das eine vom Spermakern und das andere vom Eikern herstammt (Fig. 5). Um diese Zeit degenerieren andere sieben Kerne vollständig. Also die Befruchtung bei dieser Alge erfolgt nicht im Zentrum, sondern in der Peripherie der Eizelle. Dann findet die erste Kernteilung, wie es die Meinung von TAHARA und SHIMOTOMAI ist, merkwürdigerweise immer in der Peripherie der Eizelle statt. Diesen Schluss konnte ich in einer Reihe von Präparaten bestätigen.

Fig. 8 (auch Textfig. 1, a) zeigt die Seitenansicht der früheren Metaphase der ersten Teilung des Keimkerns. Zentrosomen, die bei anderen Arten dieser Gattung (TAHARA und SHIMOTOMAI 1926, OKABE 1929, 30) entdeckt wurden, konnten bei dieser Spezies nicht beobachtet werden. Es gelang mir auch nicht, eine gute Polansicht der ersten Metaphase zu

finden, welche die diploide Chromosomenzahl von dieser Alge klar zu bestimmen erlaubt. Nach dem Eindringen des Spermakerns mussten etwa 15 Stunden verfliessen, bevor der Keimkern sich zur Teilung anschickt. Diese Zeit mag aber je nach den Umständen schwanken. In der Anaphase trennen sich die Tochterchromosomen sehr rasch auseinander

Textfig. 1, a. Metaphase der ersten Kernteilung des Keimkerns. Teilung findet in der Peripherie der Eizelle statt. b. Spätere Telophase derselben Teilung. Zwei Tochterkerne liegen in grosser Entfernung. Vergr. 360.

(Taf. X, Fig. 9). Das eine der dabei entstehenden zwei Chromosomenklümpchen bleibt gewöhnlich in der Peripherie und das andere pflegt nach innen sich zu bewegen. Also in der Telophase liegen die zwei Tochterkerne in grosser Entfernung (Textfig. 1, b).

Es ist ersichtlich, dass wir im quer oder schief durchgeschnittenen Mikrotomschnitte nur einen dieser beiden Kerne vor uns haben und leicht die Täuschung bekommen können, dass das Ei dieser Alge nur einen Kern im Zentrum habe. Meiner Meinung nach dürfte KUNIEDAS Fig. 75 einen Fall solchen Unglückes darstellen.

Wie wohl bekannt, ist es die Regel in den Fucaceen, dass im Oogonium zuerst acht Kerne entstehen. Die Zahl der Eier in einem Oogonium ist aber in den verschiedenen Gattungen dieser Familie nicht gleich, z.B. bei *Fucus* 8, bei *Pelvetia* 2 und bei *Sargassum* 1. Danach ist es zweifellos, dass das Ei

von *Sargassum* ein Verschmelzungsprodukt von 8 Eiern ist. Die Verschmel-
zung ist aber bei dieser Gattung noch nicht vollständig, weil die entleerten
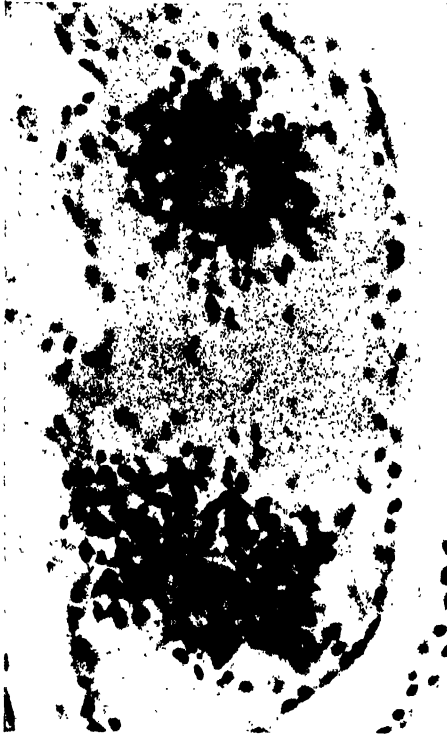
Textfig. 2. Zwei in Karyokinese befind-
liche Kerne in ein und derselben Eizelle.
Erklärung im Text. Vergr. 700.

Eier dieser Alge noch 8 Kerne in sich beherbergen, während *Coccophora*, eine andere Gattung dieser Familie, nur einen Kern im Zentrum des Eies besitzt. Die Tatsache, dass bei *Sargassum* die Befruchtung und die ihr folgende erste Kernteilung in der Peripherie des Eies sich vollziehen, mag den Gedanken über die Unvollständigkeit der Verschmelzung verstärken.

Zum Schluss möchte ich noch eine Beschreibung der abnormalen Erscheinung in dieser Alge hinzufügen. Manchmal konnte ich zwei in Karyokinese befindliche Kerne in ein und derselben Eizelle ersehen. Textfig. 2 zeigt, dass sich der eine in Prophase und der andere in Metaphase befinden. Die beiden dürften allem Anschein nach diploid sein. Also liegt die Vermutung ziemlich nahe, dass von solchen Eiern die Doppelembryonen, wie sie TAHARA (1927) seinerzeit abgebildet hat, entstehen.

Es ist mir ein Bedürfnis, an dieser Stelle meinem hochverehrten Lehrer, Herrn Prof. Dr. M. TAHARA, meinen besten Dank auszusprechen für die Anregung zu dieser Arbeit.

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TAFELERKLÄRUNG.

Alle Mikrophotographien wurden mit Hilfe von LEITZ-Makam aufgenommen: Vergr. 700.

TAFEL 1.

Fig. 1. Der Spermakern in der Nähe vom Eikern.
Figg. 2–4. Die Verschmelzung der beiden Kerne.
Fig. 5. Der Ruhekern nach der Befruchtung.
Fig. 6. Die frühere Prophase der ersten Teilung des Keimkerns.
Fig. 7. Die spätere Prophase derselben.
Fig. 8. Die Seitenansicht der frühere Metaphase.
Fig. 9. Anaphase.

BEOBACHTUNGEN EINIGER THIOTROPHER SEEN JAPANS MIT BESONDERER BERÜCKSICHTIGUNG DER SCHWEFELBAKTERIEN

Von

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(Mit drei Textfiguren)

(Eingegangen am 20. Oktober 1938)

Der thiotrophe See im Sinne Decksbachs ('31) kennzeichnet sich dadurch, dass die fortwährend oder periodisch stagnierende untere Wasserschicht den Sauerstoffschwund und zugleich die Schwefelwasserstoffanhäufung zeigt.[1]

Aus den bisherigen Forschungen lassen sich seine *allgemeinen Beschaffenheiten* folgendermassen zusammenfassen :

1) Zur starken Schwefelwasserstoffanhäufung ist die Stagnation in hohem Grade, neben der heftigen Entwicklung dieses Gases, unbedingt notwendig.

2) Im ausgeprägt thiotrophen See besteht die immer stagnierende Wasserschicht in der Tiefe entweder aus dem Meerwasser (an der Meeresküste) oder aus dem von der unterseeischen Quelle herstammenden Salzwasser (im Binnenlande), wegen dessen Schwere diese Wasserschicht unabhängig von der Zirkulation in der darüber lagernden süsseren Wasserschicht steht. Freilich muss die Diffusion der gelösten Substanzen voneinander an der Grenzschicht einigermassen stattfinden

3) Unter den Brackwasserseen ist der Grad der Stagnation und dementsprechend auch der Schwefelwasserstoffgehalt allerhöchst in denjenigen, welche sich jemals vermutlich beim Sturme dem Einflusse des Meerwassers unterzogen haben und sonst ohne Verbindung mit dem Meere stehen, sodass das unten lagernde Meerwasser seitdem in Ruhe bleibt. Diese extremen Fälle sind von dem Harutori See (Japan) und Hemmelsdorfer See (Deutschland)[2] vertreten. Insbesondere zeigt der erstere einen un-

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[1] Auch die Bucht, vor allem der norwegische Fjord; mit einem seichten Eingange kann gleichfalls thiotroph sein. Ein längst bekanntes Beispiel davon ist das Schwarze Meer. [Vgl. Issatchenko ('24), Schokalsky und Nikitine ('27), Strøm ('36) und Bavendamm ('24), S. 36-37.]

[2] Siehe Griesel ('21).

geheueren Schwefelwasserstoffreichtum wie über 600 mg/l, der gegenüber den letzteren zweifach konzentriert ist.

4) Der grösste Schwefelwasserstoffgehalt (786 mg/l) ist neuerdings von HUTCHINSON ('37) bei Big Soda Lake, ein Salzsee im Westen der Vereinigten Staaten (Nevada), ermittelt worden.[1]

5) Das schwefelwasserstoffhaltige Tiefenwasser, sowohl als der an dieses angrenzende Seeboden, sind azoisch, abgesehen von gewissen Mikroben. Darunter haben die Purpurbakterien, z. B. Chromatien, vielfach die Aufmerksamkeit der Limnologen angezogen. Sie schweben mit Vorliebe an der oberen Grenze der schwefelwasserstoffhaltigen Wasserschicht, manchmal so scharenweise dass das Wasser rötlich gefärbt wird. Ausserdem sind noch farblose Achromatien ihrer Riesigkeit halber zuweilen gefunden worden, und sie sammeln sich an der Grenzschicht ebenso wie die Purpurbakterien an. Ausser bei uns ist diese Erscheinung beobachtet worden: beim Hemmelsdorfer See und Edebergsee (Deutschland) von UTERMÖHL ('25)[2], beim Ritom See (Schweiz) von EDER-SCHWYZER ('24) und DÜGGELI ('24), beim Rotsee (Schweiz) von DÜGGELI ('19) und BACHMANN ('31), beim Cadagno See (Schweiz) von DÜGGELI ('24), beim Ranau See (Sumatra) von RUTTNER ('31 b) und beim Krottensee (Österreich), Toplitzsee (Österreich) und Lunzer Obersee (Österreich) von RUTTNER ('37). Die dabei gefundenen Schwefelbakterienarten sind: *Chromatium Okenii, Chromatium Weissei, Chromatium minutissimum* (?), *Thiopedia rosea, Lamprocystis reseo-persicina, Macromonas mobilis* (=*Achromatium mobile*) und *Macromonas bipunctata*.

Hier sind *thiotrophe Seen Japans* genannt, deren Areale, grössten Tiefen und bisher bestimmten grössten Schwefelwasserstoffgehalte[5] in Klammern darauf folgen: der Harutori See (0,5 km², 9 m, 675 mg/l); der Suigetsu See (5 km², 34 m, 61 mg/l); Namakoike (0,5 km², 22 m, 40 mg/l); der Hiruga See (1 km², 39 m, 37 mg/l[1]); Kaiike (0,2 km², 12 m, 26 mg/l); Shinmiyo[5] (0,06 km², 35 m, 20 mg/l); der Hamana See (72 km², 16 m, 14 mg/l); Onnetô (0,8 km², 22 m, 14 mg/l); Mokotonuma[1] (1 km², 6 m, 12 mg/l); Yosanaikai (5 km², 14 m, 5 mg/l); Koyamaike (7 km², 9 m,

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[1] In bezug auf andere fremde thiotrophe Seen, vgl. NADSON ('03), BOURCART ('06), EDER-SCHWYZER ('24), DÜGGELI ('19, '24), DELEBECQUE ('98), BACHMANN ('31), OHLE ('34), RUTTNER ('31 a, b; '37) und BAVENDAMM ('24).

[2] Vgl. auch OHLE ('34).

[3] In bezug auf die Autoren, usw., siehe Ecol. Rev. ("Seitaigaku-kenkyû"), 4, 253, 1938. Siehe auch die übersichtliche Schilderung einzelner Seen von YOSHIMURA ('38 b).

[4] Nach einem rezenten amtlichen Berichte.

[5] Vgl. YOSHIMURA und MIYADI ('36).

[6] Vgl. UÉNO ('38).

4 mg/l); Wakuike[1] (0,03 km², 11 m, 19 mg/l). Wakuike ist der einzige Salzsee, während alle anderen zu den Brackwasserseen zu rechnen sind.

Unter den oben angeführten, sind der Harutori, Suigetsu und Hamana See näher und auch betreffs Schwefelbakterien untersucht worden, und auch ich hatte im letzten Sommer Gelegenheit sie zu besuchen. Deswegen will ich im folgenden darüber eingehend sprechen.

DER HARUTORI SEE

Der Harutori See an der südöstlichen Küste von Hokkaidô ist schmal, etwas zickzackig und vollständig, ein Ende ausgenommen, in ein Plateau

TABELLE 1.
Der Harutori See.

(Die Querlinien zeigen die Grenzschicht und die punktierten den Seeboden, was auch für andere Tabellen gilt.)

Forscher	TAKAYASU ('33)					KUSUKI ('37)						YOSHIMURA ('37)	JIMBO		
Datum	7. VII. '32			13. X. '32		10. V. '36			13. VII. '36			4. IX. '36	7. IX. '38		
Tiefe m	P_H	Temperatur °C	H_2S mg/l	P_H	Temperatur °C	H_2S mg/l	P_H	Temperatur °C	H_2S mg/l	P_H	Temperatur °C	H_2S mg/l	H_2S* mg/l	P_H	Temperatur °C
0	9,8<	20,5	0	8,5	14,2	0	9,1	10,5	0	8,6	16,7		0	7,2	23,3
1	9,8<	20,3	0	8,6	14,1	0	9,1	10,5	0	8,6	16,7				23,0
2	9,6	18,5	0	8,6	13,8	0	9,1	10,5	0		16,5		0	7,2	22,9
3	7,1	13,3	137	7,2	14,2	0	9,1	10,4	0	8,5	16,3				22,9
4	7,1	9,4	344	7,1	12,0	31	7,4	9,4	0	8,5	16,0		0	7,2	22,8
4,5						339			171	7,2	14,7		55	8,2	18,3
5	7,1	8,3	377	7,1	10,0	440	7,0	7,3	345		12,5		296	8,4	13,0
6	7,1	8,0	378	7,1	8,9	663	7,0	7,8	636	6,9	11,7		559	8,6	10,0
7			380	7,1	8,5	675	7,0	8,5	656		11,0		578		8,7
8	7,1	8,2	344	7,1	8,2	671	7,0	9,0	664	6,9	10,2	670	621		8,5
8,5									663		9,0			8,6	8,5
9															8,4
9,5															

* Ich führte das ganze Verfahren der jodometrischen Schwefelwasserstoffbestimmung im Boote durch. Direkt von einem EKMANschen Schöpfer mit einer Spritze entnommenes Wasser wurde in n/100-Jodlösung gegossen, darauf titrierte ich mit n/100-Thiosulfatlösung zurück.

[1] Vgl. YOSHIMURA ('36).

eingeschlossen. Das offene Ende ist durch einen Strand mit einer Reihe von Fischerhäusern vom Meere getrennt ohne irgendeinen Zusammenhang. Anscheinend war der Wasserspiegel des Sees bedeutend höher als jener des Meeres, und die immer zunehmende Wassermenge wird etwa monatlich künstlich ins Meer weggegossen. Den bisherigen Untersuchungen nach misst der Chlorgehalt über 1 g/l in der oberen Wasserschicht und über 13 g/l in der unteren.
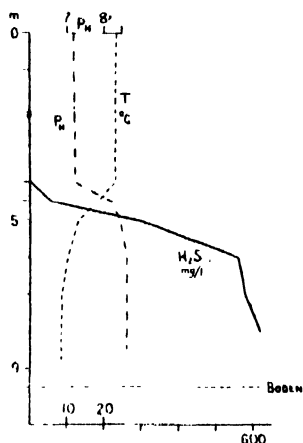
Abb. 1. Der Harutori See.

Die eigenen Ergebnisse sind in Tabelle 1 und Abbildung 1 gezeigt, worin auch diejenigen der anderen Forscher zum Vergleiche wiedergegeben sind.

Es ist auffallend, dass meine Resultate insofern ganz verschieden von denen der anderen sind, als die stagnierende Wasserschicht deutlich alkalisch war, während sie andernfalls immer fast neutral gewesen war. In Wirklichkeit liess es sich ermitteln, dass das Wasser dieser Schicht den scharfen Geruch und stechenden Geschmack von freiem Ammoniak hatte.

Ausserdem fand ich gegen alle Erwartungen keinerlei Purpurbakterien darin. Trotzdem die auf Chromatienentwicklung beruhende rötliche Färbung der Grenzschicht dabei schon von Yoshimura ('37) und Hada ('38) wiederholt beobachtet worden ist, waren die ganzen Wasserschichten farblos beim Schöpfen und wurden sie beim Aufbewahren nie rötlich. Ferner wurde die Abwesenheit von Purpurbakterien darin bei mikroskopischer Betrachtung festgestellt. Es liegt nahe, dass das Ammoniak bewirkt hatte diese Bakterien wegzutreiben.

Es fragt sich nun, wie die ausserordentliche Ammoniakanhäufung verursacht wurde, und ob nicht dieses Phänomen vorübergehend war. Allerdings ist eine entscheidende Antwort dazu durch fortlaufende Untersuchung zu bekommen, doch sind wir berechtigt aus damaligen Verhältnissen die folgende Mutmassung zu tun: Wenn wir zwei Tatsachen berücksichtigen, erstens dass ich zufälligerweise gleich nach Sturme die Beobachtung machte, und zweitens dass, zu meinem Erstaunen, der Anker immer beim Ausnehmen vom azoischen Seeboden mit einem grossen Masse der am Ufer massenhaft wuchernden Wasserpflanzen, die ziemlich frisch aussahen, geladen wurde, ist es überaus wahrscheinlich, dass die starke

Ammoniakentwicklung die Folge der auf dem Seeboden heftig geschehenden Fäulnis[1] der beim Sturme entwurzelten und zu der Tiefe gebrachten Wasserpflanzen war. So scheint diese Erscheinung ganz vorübergehend zu sein und vielleicht begegnete ich unerwartet einem anomalen Falle.

Alle bisherigen drei Forscher sind der Meinung gewesen, dass der Schwefelwasserstoff in diesem See das Reduktionsprodukt der im Meerwasser von vornherein enthaltenen Sulfate ist. Aber wir können die gleichzeitige Entwicklung einer beträchtlichen Menge von Schwefelwasserstoff nicht verneinen, falls Ammoniak nach dem Sturme aus pflanzlichem Eiweissstoff so gewaltig produziert wird. Jedenfalls nun dass die Sulfate in der Tiefe erschöpft sind, was KUSUKI und YOSHIMURA ermittelt haben[2], muss der Verlust von Schwefelwasserstoff namentlich von der Grenzschicht vorzüglich derart ersetzt werden. Die die Herkunft des Schwefelwasserstoffs betreffende Frage wird erst dann gelöst werden, wenn die zeitliche Schwefelwasserstoffkurve besonders bei einem solchen Sturme gründlich untersucht worden sein wird. In der Tat ist das Schicksal des Schwefelwasserstoffs dieses Sees eine sehr interessante Aufgabe.

Bevor wir zu der Schilderung der anderen Seen übergehen, wollen wir auf die *die Herkunft des Schwefelwasserstoffs in den verschiedenen thiotrophen Seen* betreffenden vorherrschenden Ansichten und die diesbezüglichen Tatsachen einen Blick werfen:

YOSHIMURA ('31, '32, '34, '37) hat behauptet, dass der Schwefelwasserstoff in den thiotrophen Brackwasserseen gewöhnlich von den im Meerwasser gelösten Sulfaten herstammt, auf Grund seines Befundes wie folgt: Wenn man das Verhältnis der Süss- und Meerwasser aus dem Chlorgehalte jeder Wasserschicht und daraus die Sulfatenmenge berechnet, ist die empirisch festgestellte Sulfatenmenge bedeutend weniger als die berechnete in den unteren Schichten während in den oberen Schichten alle beide im Einklang stehen. Weiterhin ist auch der empirische Wert des Schwefelwasserstoffs geringer als der aus der Differenz der berechneten und empirisch erhaltenen Sulfatenmengen umrechnete Wert. Besonders im Harutori See stimmen die beiden Werte überein, was auch von den Resultaten der anderen beiden Forscher gilt. In Wirklichkeit, neben den Brackwasserseen, deren Sulfatenreichtum selbstverständlich ist, sind auch die binnenländischen thiotrophen Seen, wie Wakuike[3], der Ritom See[4], der Cadagno See[5] und die von RUTTNER ('31 a) untersuchten tropischen Seen, sulfatenreich; und überdies sind die sulfatreduzierenden Bakterien aus der unteren Wasserschicht des Ritom Sees und den Böden dieses Sees[6], des Suigetsu Sees[7] und des Schwarzen

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[1] Dass die Fäulnisbakterien im Bodenschlamm der thiotrophen Seen und Buchten leben, ist beim Ritom See und beim Schwarzen Meere festgestellt worden. [Vgl. DÜGGELI ('24), ISSATCHENKO ('24) und GINSBURG-KARAGITSCHEWA und RODIONOWA ('35).]

[2] Dagegen fand TAKAYASU vier Jahre vorher eine grosse Menge von Sulfaten bis zu ungefähr 800 mg/l SO_3 auch in den tiefen Wasserschichten.

[3] Vgl. YOSHIMURA ('36).

[4] Vgl. BOURCART ('06).

[5] Vgl. BOURCART ('06).

[6] Vgl. DÜGGELI ('24).

[7] Vgl. KIMATA ('38).

Meeres[1] isoliert worden. Daraus haben DÜGGELI ('24) und ISSATCHENKO ('24) den Schwefel-
wasserstoff des Ritom Sees und Schwarzen Meeres als das Reduktionsprodukt der Sulfate
angesehen.

Demgegenüber ist es wahrscheinlich, mindestens bei den gewöhnlichen Süsswasserseen,
dass organische Substanzen die wichtigste Quelle des Schwefelwasserstoffs sind. Zwar ver-
dankt der Rotsee bei Luzern, DÜGGELIs ('19) Ansicht nach, den Schwefelwasserstoff den
von dieser Stadt einfliessenden Abwässern[2]. Ferner hat OHLE ('34) in norddeutschen Süss-
wasserseen grössere Schwefelwasserstoffanhäufung dortin bemerkt, wo der Grad der Eutrophie
höher ist. Auch in bezug auf die thiotrophen Brack- und Salzwasserseen sowie Buchten,
muss man das Vorhandensein der Fäulnisbakterien in ihrem sapropelischen Bodenschlamm,
wie schon gesagt, und die vielfach festgestellte Anhäufung von Kohlensäure, Ammoniak und
Phosphorsäure, die als die Zersetzungsprodukte organischer Substanzen anzunehmen sind,
besonders in ihrer tiefsten Wasserschicht berücksichtigen.

DER SUIGETSU SEE

Der Suigetsu See an der Wakasa Bucht ist rundlich und von hohen
steilen Hügeln umgegeben. Dieser tiefe und stille See steht von alters
her durch einen Kanal einerseits und durch einen Tunnel anderseits in
Verbindung mit dem Kugushi See und dem Hiruga See, die ihrerseits
direkt mit dem Meere zusammenhangen.

Wenn auch der Schwefelwasserstoff im immer stagnierenden Tiefen-
wasser dieses Sees bei mehrmaligen Untersuchungen der provinzialen
Versuchsstation für Fischerei, sowie von MATSUDAIRA ('29), KIKUCHI ('30,
'31) und YOSHIMURA ('34) bestimmt worden ist, bleibt Näheres über die
von KIKUCHI an der Grenzschicht gefundenen „Schwefelbakterien" un-
bekannt.

Das hydrographische Verhältnis bei meinem Besuche ist in Tabelle 2
und Abbildung 2 veranschaulicht.

Wenn ich eine Reihe mit einem EKMANschen Schöpfer entnommener
Wasserproben verglich, wurde schwache aber deutliche rote Farbe bei
7 m Tiefe wahrgenommen, die eben die oberste Wasserschicht mit analy-
tisch erkennbarem Schwefelwasserstoff ist. Auch bei 6,5 m Tiefe konnten
schwächere, und bei 6 m, 7,5 m und 8 m Tiefen immer schwächere Tink-
turen erkennt werden, während die Wasser aus 5,5 m und 9 m Tiefen,
sowie beziehungsweise darüber und darunter lagernde Wasserschichten
durchaus farblos waren. Sicherlich musste der Schwefelwasserstoff noch
bei 6 m Tiefe in einer ganz geringen Menge sich vorfinden[3].

[1] Vgl. ISSATCHENKO ('24) und GINSBURG-KARAGITSCHEWA und RODIONOWA ('35).
[2] Siehe hierüber auch BACHMANN ('31).
[3] Indessen konstatierte ich die ebenso gefärbte Grenzschicht bei ganz derselben Tiefe
wie beim Suigetsu See auch im mit diesem See zusammenhangenden Suga See (ca. 13 m
tief).

TABELLE 2.
Der Suigetsu See.

Datum	19. VIII. '38		
Tiefe m	H_2S mg/l	P_H	Temperatur °C
0	0	6, 9	28, 5
2, 5	0		28, 2
5	0	7, 0	22, 0
6	0		
6, 5	0		
7	2		
7, 5	4		17, 8
8	8		
10	14	7, 2	16, 8
15		7, 1	16, 7
20	15	7, 1	16, 8
25		7, 1	17, 2
27, 5	32		
30	35	7, 5	17, 4
32	41		
33			17, 4
33, 5			
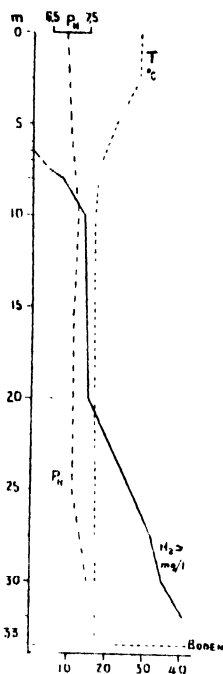
Abb. 2. Der Suigetsu See.

Bei unverzüglicher Untersuchung des gefärbten Wassers aus der Grenz-schicht fand ich darin zweierlei Chromatien, die mit *Chromatium minus* WINOGRADSKY und *Chromatium minutissimum* WINOGRADSKY identisch sind[1]. Nebst diesen schwefelführenden Purpurbakterien nahm ich noch eine schwefelfreie Purpurbakterie wahr. Diese ist von kurzer Stäbchen-form, höchstens 0,5 μ dick, erscheint orange-rot und bildet kleine in Gallerte zusammengedrängte Massen. Es schien, dass die Grenzschicht ihre rote Farbe vornehmlich diesem roten Stäbchen verdankte.

DER HAMANA SEE

Der Hamana See ist sehr ausgedehnt, aber ziemlich seicht, und steht im unmittelbaren Zusammenhang mit dem Meere. Dieser See unter-scheidet sich von den vorhergehenden, sofern die Vollzirkulation hierbei geschiet und daher sich die Schwefelwasserstoffanhäufung nur bei der

[1] Vgl. WINOGRADSKY ('88), S. 99 100.

Sommerstagnation findet. Hydrographische Untersuchungen dieses Sees sind von der dortigen Versuchsstation für Fischerei und provinzialen meteorologischen Station ('37) durchgeführt worden[1].

INABA ('36) von der Versuchsstation für Fischerei hat eine scharenweise Entwicklung von Chromatien an der oberen Grenze der schwefelwasserstoffhaltigen Wasserschicht ermittelt, und dadurch wurde das Wesen der im Herbste die stürmische Witterung begleitenden und infolge ihrer schädlichen Wirkung auf Fische von Fischern gefürchteten „roten Gezeit" klargemacht[2].

Ich ging dorthin gegen Mitte August, zur Zeit wo der See noch nicht die Höhe der Stagnation erreicht hatte. Daher, wie aus Tabelle 3 und Abbildung 3 zu ersehen ist, war die Schwefelwasserstoffkonzentration sehr niedrig und überdies lag die Grenzschicht bei 10 m Tiefe, obgleich sie

TABELLE 3.

Der Hamana See.

Datum	16. VIII. '38		
Tiefe m	H_2S mg/l	P_H	Temperatur °C
0	0	7,0	30,0
1		6,9	29,3
2	0	6,9	28,9
3		6,8	27,3
4		7,0	26,3
5	0	7,1	25,3
6		7,1	24,7
7		7,0	24,3
8	0	7,2	24,0
9	0	7,1	23,3
10	1	7,1	22,7
11	1	7,2	22,4
12	2	7,1	21,8
13	4	7,2	21,7
14	5	7,2	21,7
14,5			
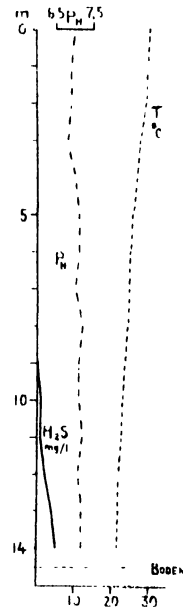
Abb. 3. Der Hamana See.

[1] Vgl. auch YOSHIMURA und WADA ('38).

[2] Gleichfalls erfahren die am Ufer des Suigetsu Sees wohnenden Leute zuweilen beim Sturme den grossen Schaden der Fische durch emporsteigenden Schwefelwasserstoff, was auch bei fremden Seen nicht selten ist.

gegen Oktober sogar bis zu 6 m Tiefe hinauf zu erreichen ist. Leider fand ich keine Farbe bei der Grenzschicht. Dennoch, die in Fläschchen unter Luftabschluss aufbewahrten Wasser aus den verschiedenen schwefelwasserstoffhaltigen Schichten wurden nach wenigen Tagen rötlich, was auf einer merklichen Entwicklung von Purpurbakterien beruhte. Es ist bemerkenswert, dass dabei der Färbe-Grad mit der Tiefe, aus der das Wasser entnommen wurde, zunahm ; mit anderen Worten war die Vermehrung der Purpurbakterien im gleichen vollen Lichte proportional mit der Schwefelwasserstoffkonzentration. Mit der Tatsache, dass ebenfalls das Wasser aus der tiefsten Schicht des Suigetsu Sees beim Aufbewahren sich rötlich verwandelte, führt diese Erscheinung einen Beweis dazu, dass das Zusammenkommen der Purpurbakterien an der Grenzschicht in den thiotrophen Seen ausschliesslich der Phototaxis zuzuschreiben ist. Vermutlich hängte das Fehlen der roten „ Bakterienplatte " an der Grenzschicht an Ort und Stelle vornehmlich von der unzulänglichen Schwefelwasserstoffzufuhr ab. In Wirklichkeit färbte sich sogar das Wasser aus der Grenzschicht beim Aufbewahren im Fläschchen im vollen Lichte, jedoch ist es nicht unwahrscheinlich, dass sich mehr oder weniger Schwefelwasserstoff dabei auf irgendeine Weise entwickelte.

In den roten Wassern in den Fläschchen habe ich dasselbe schwefelfreie rote Stäbchen wie im Suigetsu See, sowie *Chromatium minus* gefunden. Daneben fanden sich noch Chromatienzellen, welche ebenso dick wie die von *Chromatium Weissei* aber viel kürzer als sie waren ; das Nähere davon habe ich noch nicht klargelegt.

ZUSAMMENFASSUNG

1. Beim Suigetsu See, unter drei von mir untersuchten Brackwasserseen mit schwefelwasserstoffhaltigem Tiefenwasser, wurde die „ Purpurbakterienplatte " an der oberen Grenze der schwefelwasserstoffhaltigen Wasserschicht beobachtet. Diese bestand vornehmlich aus einem schwefelfreien roten Stäbchen, das von *Chromatium minus* und *Chromatium minutissimum* begleitet wurde.

2. Dasselbe rote Stäbchen wie oben sowie *Chromatium minus* fanden sich auch in den sich beim Aufbewahren in Fläschchen rötlich verwandelten Wassern aus verschiedenen schwefelwasserstoffhaltigen Schichten des verhältnismässig labilen Hamana Sees, obgleich die rote Bakterienplatte wegen unzureichender Schwefelwasserstoffanhäufung noch nicht gebildet war.

3. Beim äusserst schwefelwasserstoffreichen Harutori See, wo ich nach einem Sturme einen Besuch machte, wurde eine enorme Ammoniak-anhäufung und demzufolge Abwesenheit von Purpurbakterien ermittelt. Vermutlich entwickelte sich das Ammoniak von beim Sturme zu der Tiefe gebrachten faulenden Wasserpflanzen. Gleichzeitige starke Entwicklung von Schwefelwasserstoff ist überaus wahrscheinlich.

Zum Schluss sage ich Herrn Dr. S. YOSHIMURA für seine gütige Unterstützung den wärmsten Dank, desgleichen auch Herrn Y. SAKUMA, welcher bei der Feldarbeit einen unschätzbaren Dienst leistete.

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ON THE DISTRIBUTION OF *TRICHODERMA* IN THE SOILS OF VARIOUS TYPES OF VEGETATION ON MT. HAKKODA*

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I. INTRODUCTION

It is a fundamental knowledge in plant sociology nowadays that plants establish their appropriate communities in accordance with their environmental conditions. The same principle doubtless holds good for soil microbes as well; they build up, in the soil, their proper communities, which at the same time, may be regarded as a part of the whole vegetation. However, the majority of plant sociological studies hitherto have been limited to higher plants, especially to seed plants and vascular cryptogams. It has been extremely rare that fungi, even the larger forms, have been treated as the participants in the construction of the plant community. To a still greater extent is such the case with the microscopic forms. Only a limited number of papers are found which deal with the relation of some groups or species of microbes to the phanerogamic vegetation. WENZL[7] studied the distribution of *Azotobacter chroococcum* with respect to the types of phanerogamic communities found in the region near Lake Neusiedl. In his paper are also given references to earlier works on cognate matter, which need not be cited here. Recently K. HÖFLER[2] proposed applying the usual method of plant sociology to the analytical study of communities of fungi, and gave some examples of his study concerning those in beech forests.

During the past few years, the present author made some quantitative studies of the soil microflora in the *Pseudosasa kurilensis*-community on Mt. Hakkoda[1]. In the course of these studies, he occasionally noticed the almost constant occurrence of *Trichoderma*[5]. Now, this mold represents, as is generally recognized, one of the strongest cellulose-decomposing agents in the soil, and it seems likely that this species is quite

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important in this respect in the *Pseudosasa*-community. So, while staying at the Mt. Hakkoda Botanical Laboratory in the summers of 1936 and 1937, the author tried to study the distribution of this mold in the *Pseudosasa* community as well as in other communities there. In the following paragraphs are reported the results of these investigations.

II. METHODS

The plating method is generally accepted as the most convenient means for studying the number of microbes in the soil. The writer, too, had recourse chiefly to this method in his previous study of the soil microflora of the *Pseudosasa* community. However, as the mountain laboratory is not specially equipped for microbial studies, the convenience of working with simpler methods was keenly felt, especially when a large number of materials were at hand. Therefore, by way of experiment, the writer tried streak cultures on nutrient agar plates directly from the soil. The general outline of the operation was as follows. As a culture medium, WAKSMAN's peptone-glucose acid agar[1] was employed. About 15 ml. of this medium were poured into a sterile petri dish, and coagulated and kept at 27° to 28°C for 4 to 5 days to verify its sterility. The plates, packed in a sterilized metal container, were subsequently taken to the field of experiment, where direct streaks were made from a freshly exposed soil surface, by means of a nichrome wire of sufficient strength (nichrome wire about 0.5 to 1 mm. wide and having its end flattened into a chisel shape was found quite expedient for the purpose). Seven streaks were drawn on each plate, their length and position being kept constant throughout the experiment. For each plot of field of study, at least 5 points (sometimes 10 points) with a few meter intervals were chosen and one plate was prepared from each point, so that 5 plates (35 streaks) or sometimes 10 plates (70 streaks) were prepared for each field of study. These finished plates were brought directly to the laboratory, kept at 27° to 28°C, and examined daily in order to count the fungal colonies developing on them. At first the colonies kept on growing with their original circular outline, but shortly they began to fuse into each other, making it quite difficult to distinguish their individuality simply by their appearance. Consequently the examinations had to be carried on as often as possible. After about seven days, it was almost impossible to discern the freshly developing colonies, for which reason, the examinations were then closed, and the counts up to this day were adopted as the

basis of comparison for the respective test fields.

One serious defect of this method, besides the above mentioned difficulty in discerning the individuality of each colony, is that the slowly growing species are apt to be covered by rapidly growing ones, which circumstance makes the application of the method quite limited. However, for the purpose of the present study, which mainly concerns the presence or the absence of *Trichoderma*, the method is very convenient as the mold in question is characterized by a very rapidly growing habit. As for the purpose of estimating the number of the mold in the soil, the streak method is entirely inadequate to give the absolute number. Still, some idea may be obtained on the relative abundance of this mold in the soil by standardizing the procedure of the operation.

On the other hand, the merit of the streak method may also be mentioned. It is found in the simplicity of the operation which minimizes the danger of contamination from aerial germs. The plating method, in which a rather complicated procedure of dilution is imperative, is much more open to the danger of aerial contamination as compared with the streak method.

In brief, the streak method is not at all an excellent one to use in place of the plating method. However, as the latter method, too, is not absolutely perfect, there is some reason, under certain circumstances at least, for the application of the former. Especially, when it is the kind, and not the number of the soil microbes that is of chief concern, the streak method may well be recommended.

In the present study, the distribution of *Trichoderma* in the soil of various types of vegetation was examined by means of the streak method, and in some cases, it was used also with respect to the depth of the soil. With some soils, the comparison with the value obtained by the plating method was made. A few experiments were also carried out as to whether this mold exists in the soil as a vegetative body or as a spore. In this last case, the usual method of plating was applied.

III. THE FIELDS OF EXPERIMENT

Soils from the following nine types of vegetation were subjected to study, viz., 1) Pseudosasetum, 2) Fagetum, 3) Abietetum, 4) Pinetum, 5) Bare land around the crater, 6) Bare land near the solfatara, 7) Cladonietum near the solfatara, 8) Sphagnum moor and 9) Narthecietum. The altitude of the test fields, the depth of the soil sampled, its water

content, the loss on ignition, its pH-value, etc., are all shown in the accompanying table.

TABLE I

Type of vegetation	Altitude (m.)	Depth of the soil (cm.)	Water content of the soil (%)	Loss on igni-tion (% on the basis of dry weight	pH-value	Type of the soil
1. Pseudosasetum	900	10-20	69.2	32.8	4.7	raw humus
2. Ditto	,,	25	53.5	18.6	5.1	clayey soil with raw humus
3. Ditto	,,	40	61.8	20.6	4.9-5.1	clayey soil
4. Ditto	,,	80	50.2		5.1	clayey soil
5. Fagetum	780	10-20	62.2	23.7	4.1-4.2	raw humus
6. Abietetum	980	10-20	59.2	29.6	3.7	raw humus
7. Pinetum	1540	6-10	74.4	64.3	3.7	raw humus
8. Bare land around the crater	1550	6-10	9.0	3.0	5.4	sandy soil
9. Bare land near the solfatara	950	1-3	26.5	17.7	2.0-2.2	sandy soil
10. Cladonietum near the solfatara	950	1-3	46.6	14.9	2.8-3.1	sandy soil with raw humus
11. Sphagnum moor	980	20*	90.4	76.0	3.7	peat
12. Narthecietum	980	20*	88.4	61.0	3.7	peat

* measured from the top of the sphagnum layer.

The compositions of the plant communities of these test fields are** :

1) **Pseudosasetum.** 5 2 m. *Ilex Sugeroki* subsp. *brevipedunculata* **1**, *Acanthopanax sciadophylloides* +, *Sorbus commixta* +, *Acer Tschonoskii* + ; 2-1 m. *Pseudo-sasa kurilensis* **5**, *Rhus trichocarpa* +, *Viburnum furcatum* +, *Acer Tscho-noskii* + ; 100-10 cm. *Ilex leucoclada* +, *Viburnum furcatum* +, *Ilex Suge-roki* subsp. *brevipedunculata* +, *Daphniphyllum glaucescens* + ; 10 cm. *Maianthemum bifolium* +, *Ilex Sugeroki* subsp. *brevipedunculata* + ; climbers *Rhus toxicodendron* var. *radicans* +, *Crawfurdia japonica* +.

2) **Fagetum.** 5 m. *Fagus crenata* **5** ; 5-2 m. *Acer japonicum* var. *typicum* **1** ; 2 1 m. *Pseudosasa kurilensis* **2**, *Magnolia salicifolia* +, *Acer japonicum* var. *typicum* +, *Cephalotaxus nana* + ; 100-10 cm. *Ilex leucoclada* +, *Strepto-pus japonicus* +, *Viburnum furcatum* +, *Acanthopanax sciadophylloides* +.

3) **Abietetum.** 5 m. *Abies Mariesii* **5** ; 5-2 m. *Acer Tschonoskii* **2**, *Fagus crenata* **2**, *Abies Mariesii* **2** ; 2 1 m. *Pseudosasa kurilensis* **4**, *Acer Tschonoskii* **1**, *Abies Mariesii* **1** ; 100-10 cm. *Viburnum furcatum* **1**, *Acer Tschonoskii* **1**,

** The figures in heavy type indicate the cover degree (Deckungsgrad) of each species, after the so-called HULT-SERNANDER scale, viz., 5 100-50%, 4 50-25%, 3 25-12.5%, 2 12.5-6.25%, 1 less than 6.25%, + sparse.

Abies Mariesii 1, *Platanthera aphrydioides* +, *Plagiogyria Matsumuraeana* +; <10 cm. *Abies Mariesii* 1, *Maianthemum bifolium* 1, *Monotropa uniflora* var. *pentapetala* 1, *Myrmechis japonica* +, *Asarum Sieboldi* +, musci 2.

4) Pinetum. 100–10 cm. *Pinus pumila* 5, *Sorbus commixta* +, *Rhododendron Fauriae* var. *rufescens* +, *Ilex rugosa* +, *Vaccinium Smallii* +, *Acer Tschonoskii* +; <10 cm. musci 5, *Coptis trifolia* +.

5) Bare land around the crater. Denudated.

6) Bare land near the solfatara. Denudated.

7) Cladonietum near the solfatara. *Cladonia polydactyla* 5.

8) Sphagnum-moor. 100–10 cm. *Lobelia sessilifolia* 1, *Moliniopsis japonica* 1, *Eriophorum gracile* 1; <10 cm. *Sphagnum* sp. 5, *Oxycoccos vulgaris* 1.

9) Narthecietum. 100–10 cm. *Narthecium asiaticum* 5, *Shortia soldanelloides* var. *genuina* f. *typica* 1, *Juncus curvatus* 1; 10 cm. *Sphagnum* sp. 5, *Drosera rotundifolia* 1, *Oxycoccos vulgaris* 1.

IV. ON THE SPECIES OF *TRICHODERMA*

Species of *Trichoderma* isolated from the above mentioned fields were found almost similar to each other. On the peptone-glucose acid agar medium, at about 27° to 28°C, they grow well, spread rapidly over the surface of the medium and the colonies assume a loose floccose appearance. Hyphae septate, colorless, about 3.5 μ wide. Conidiophores usually tri-furcate with a globular swelling at the end of each branch. Conidiospores elliptical, about $3.5 \times 2.5\,\mu$. Spore-bearing area of the colony appears green. From these morphological characters, it is without doubt that this mold belongs, or at least is very closely related to, *Trichoderma koningi*[1,3]. Although there were noticed slight variations in the shade of the green color in the spore bearing area, it cannot be readily deter-mined whether the difference is significant enough to distinguish species or varieties, so that, in the present paper, they are all treated as belong-ing to one species, *Trichoderma koningi*.

V. DISTRIBUTION OF *TRICHODERMA* IN THE SOILS OF VARIOUS TYPES OF VEGETATION

The results of the study of the distribution of *Trichoderma* by means of the streak method are shown in the accompanying table. It is clearly indicated that this mold is almost ubiquitous. A unique case of exception is plot no. 6, which is a soil of exceptionally high acidity (pH-value 2.0– 2.2, c.f. Table I), where neither humus nor living plants can be found*.

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* This statement cannot be absolutely applicable to microbes, which subject will be treated in a separate report.

TABLE II

	Number of plates pre-pared	Total number of fungal colonies per plate	Number of Tri-choderma colonies per plate	Percentage of Trichoderma colonies
1. Pseudosasetum	10	9.9	4.3	43
2. Fagetum	10	10.9	3.9	35.8
3. Abietetum	10	14.2	3.3	23.2
4. Pinetum	5	3.2	1.6	50
5. Bare land around the crater	5	9.6	0.4	4.2
6. Bare land near the solfatara	5	17.0	0	0
7. Cladonietum near the solfatara	5	67.6	2	3
8. Sphagnum-moor	5	1.8	0.6	33
9. Narthecietum	5	1.2	1.0	83.3

In all of the other cases, the presence of this mold was demonstrated. Although in small numbers, the mold was also found in the detritus of the bare land around the crater. In this last case, it must have originated, presumably, from the adjacent plant covered areas.

In the soils covered with certain plant communities, this mold is in-variably demonstrated. The density is large in soils rich in raw humus, and small in water-logged peaty ones. It was found also in the highly acidic soils of the Cladonietum of the solfatara. From these facts, it may be concluded that this mold thrives in soils well aerated and bears a quite high grade of acidity.

VI. COMPARISON OF THE RESULT OF THE STREAK METHOD WITH THAT OF THE PLATING METHOD

With soils of the Pseudosasetum, the bare land around the crater, and the Cladonietum near the solfatara, the total number of molds and that of Trichoderma were counted by means of the plating method also. The same culture medium as in the streak method was employed. As materials for study, three composite samples from five plots each were used for the Pseudosasetum soil, and one composite sample from five plots for each of the other two fields. For each composite sample, 5 to 6 platings were prepared. The results are tabulated below (Table III).

Thus, the order of the three fields arranged according to the abun-dance of Trichoderma is P-B-Cl. On the other hand, if the streak method is applied, the order runs P-Cl-B (c.f. Table II). So that there is dis-

TABLE   III

Type of vegetation	Total number of molds per 1 g. of dry soil	Number of Trichoderma per 1 g. of dry soil	Percentage of Trichoderma
Pseudosasetum (P)	ca. 6.9×10^3	ca. 2000	ca. 29%
Bare land around the crater (B)	ca. 19×10^3	ca. 480	ca. 2.6%
Cladonietum (Cl)	ca. 440×10^3	ca. 200	ca. 0.05%

cordance between the two methods with respect to the ranking of the bare land and the Cladonietum.   However, the difference in the number of *Trichoderma* in those two types of soil is not very significant and it is doubtful if even the plating method could clearly resolve the distinction.   When the difference is as remarkable as between (P) and (B), or between (P) and (Cl), both methods give concordant ranking.   However, as these studies were carried out with but three examples, safe conclusions may be attained only after further investigations.

VII.   ON THE VERTICAL DISTRIBUTION OF *TRICHODERMA* IN THE SOIL

The result obtained by the streak method with the soil of the Pseudosasetum is shown in the table below.

TABLE   IV

Depth of the soil (cm.)	Number of plates prepared	Total number of fungal colonies per plate	Number of Trichoderma colonies per plate	Percentage of Trichoderma colonies
10	10	9.9	4.3	43
25	5	0.8	0.3	50
40	5	1.8	1.2	66.6
70	5	1.0	0	0
80	5	0	0	0

Naturally, *Trichoderma* occur most in the uppermost layer, and can be found down to 40 cm. depth.   At the depth of 70 cm., however, this mold cannot be demonstrated, and at 80 cm., there are no fungus species at all.   In the table, the number of molds at 40 cm. appears larger than at 25 cm., the correctness of the matter being, however, open to criticism, as the method is not so exact as to distinguish such a small difference.

VIII. THE STATE OF *TRICHODERMA* IN THE PSEUDOSASETUM SOIL

In order to know the state of the mold in the Pseudosasetum soil, the effect of desiccation on the fungal number was studied. For this purpose, the soil sample was kept 2 weeks in a CaCl$_2$-desiccator, and counts of fungal colonies by means of the plating method were made before and after the desiccation. The result is shown in Table V.

TABLE V

	Number of molds per 1 g. dry soil	Number of *Tri-choderma* per 1 g. dry soil	Percentage of *Trichoderma*
Before desiccation	69×10^3	2000	29%
After desiccation	2.5×10^3	1400	57%

The number of *Trichoderma* is reduced to about 70%, the rest being eliminated by drying. The surviving portion may possibly be presumed to be in the state of conidiospore, that is, more than half of *Trichoderma* usually demonstrable on the plate are in a dormant state in the Pseudosasetum soil.

IX. SUMMARY

1) In the present paper is reported the distribution of *Trichoderma* as related to various kinds of the plant communities of Mt. Hakkoda.

2) The method of directly streaking on an agar plate from freshly exposed soil surface was applied to prove the occurrence of the mold. This method is not good for computing the number of molds in a definite quantity of the soil, but is quite satisfactory in excluding the contamination due to aerial germs and is therefore preferable in establishing the presence or the absence of definite species of mold in the soil. This method gives also a comparative value as to the density of the mold in the soil.

3) Soils of nine different vegetation types were studied, viz., a) Pseudosasetum, pH ca. 4.7, b) Fagetum, pH ca. 3.7, c) Abietetum, pH ca. 3.7, d) Pinetum, pH ca. 3.7, e) Detritus around the crater, pH ca. 5.4, f) Bare land near the solfatara, pH ca. 2.0–2.2, g) Cladonietum near the solfatara, pH 2.8–3.1, h) Sphagnum-moor, pH ca. 3.7 and i) Narthecietum, pH ca. 3.7. The soil from the bare land of the solfatara was proved to be free from *Trichoderma*. All of the other soils tested were found to

contain this mold.   It was demonstrated to occur profusely in soils rich in raw humus, and poorly in water-logged soils.

4) In the soils of Pseudosasetum, *Trichoderma* was found as far as in the 40 cm. deep layer, but not in the 70 cm. one.

5) *Trichoderma* in the soil of Pseudosasetum seems to exist mostly as spores.

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REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY
33. ACTINIARIA OF MUTSU BAY*

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(With Plate XI and thirty figures)

(Received November 7, 1938)

This paper is mainly based on an examination of specimens of actinians collected in connection with the biological survey of Mutsu Bay carried out during the summers of 1926 and 1927, and partly on specimens obtained from time to time by Profs. S. HôZAWA and S. KOKUBO, and Messrs. H. SATô and I. NONAKA. Observations of living specimens of some common species were made during the present writer's stay at the Asamushi Marine Biological Station in the summers of 1925, and 1927. These species being common in other localities of Japan, they have together with some from these other localities been subjected to careful comparative investigation during the past ten years. The collection includes 13 species belonging to 10 genera and 7 families. Among them, the large actinians *Anthopleura stella* and *Anthopleura japonica* are commonly found on rocks and between crevices near the tidal lines; *Anthopleura xanthogrammica* occurs frequently in slightly deeper portions, almost buried in mud, while several dozen small red actinians, *Anthopleura pacifica* n. sp. are often arranged in rows between fissures in rocks. *Diadumene Luciae* is in most cases found in great numbers on and beneath the shingle on the sea-shore. Several colour varieties of *Epiactis prolifera* and *Metridium sensile* var. *fimbriatum* usually form coloured sheets in shallow pools and depressions. A single specimen of the swimming actinian, *Boloceroides mc murrichi* was found in this bay. This species occurs very commonly on the southern coasts of Japan among sea-weeds, attached to or swimming about among the plants. The four abasilar species, *Peachia quinquecapitata*, *Eloactis mazelii*, *Harenactis attenuata* and *Andwakia hozawai*, were found buried in sand at levels deeper than the tidal lines, but the larvae of *P. quinquecapitata* are parasitic on several species

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of Hydromedusae. Besides the actinians above mentioned, a single speci-
men of *Neophellia mutsuensis* n. g. et n. sp. was obtained at some depth
off Urata. On reviewing the species of this survey, the actinians of the
bay are mostly representatives of those of the North Pacific, which have
hitherto been recorded as existing in North America, Canada and Alaska,
but *Andwakia hozawai*, *Anthopleura pacifica* n. sp. and *Neophellia mutsu-
ensis* n. g. et n. sp. are found only in Japan. The occurrence of *A.
hozawai* must be especially noted, because the genus *Andwakia* has
hitherto been represented only by a single species, *mirabilis*, found in
the Northern parts of Europe. *Neophellia mutsuensis* n. g. et n. sp. is
the actinian which has some external resemblances to the Phellidae but
from the internal features, must belong to the Paractiidae. *Boloceroides
mc murrichi* was found as a tropical immigrant. This species is widely
distributed in the distant tropical Pacific and Indian Oceans as far as
Zanzibar, Africa and the Suez Canal.

With the publication of this paper the writer wishes to express his
cordial thanks to Prof. S. Hôzawa, Dr. S. Kokubo and Mr. H. Satô
for putting specimens at the writer's disposal and for items of important
information about some actinian species. For the photomicrographs here
given the writer is extremely grateful to Mr. H. Yamaguchi of this
Institute. A part of the work was carried out through a grant from
the Foundation for the Promotion of Scientific and Industrial Research
in Japan.

<div align="center">

Tribe **NYNANTHEAE**

Subtribe **BOLOCEROIDARIA**

Family **BOLOCEROIDIDAE**

</div>

1) **Boloceroides mc murrichi** (Kwietniewski)

<div align="center">

Subtribe **ATHENARIA** (Abasilaria)

Family **HALCAMPOIDAE**

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2) **Peachia quinquecapitata** Mc Murrich
3) **Eloactis mazelii** (Jourdan)
4) **Harenactis attenuata** Torrey

<div align="center">

Family **ANDWAKIIDAE**

</div>

5) **Andwakia hozawai** Uchida

Subtribe ENDOMYARIA
Family BUNODACTIIDAE

6) **Anthopleura stella** (VERRILL)
7) **Anthopleura xanthogrammica** BRANDT
8) **Anthopleura japonica** VERRILL
9) **Anthopleura pacifica** n. sp.
10) **Epiactis prolifera** VERRILL

Subtribe INERMIA
Family PARACTIIDAE

11) **Neophellia mutsuensis** n. g. et n. sp.

Subtribe ACONTIARIA
Family DIADUMENIDAE

12) **Diadumene Luciae** (VERRILL)

Family METRIDIIDAE

13) **Metridium sensile** var. **fimbriatum** VERRILL

Subtribe BOLOCEROIDARIA
Family BOLOCEROIDIDAE

Boloceroides mc murrichi (KWIETNIEWSKI)

Boloceroides mc murrichi: CARLGREN, 1900, pp. 16-18; —. 1924, p. 16; UCHIDA, 1938,
    Ann. Zool. Jap., vol. 17, pp. 623-635.
Boloceroides sp.: OKADA, 1930, pp. 708-713; KOMORI, 1931, pp. 55-62, pl. 4; OKADA
    et KOMORI, 1932, pp. 164-199;
Bolocera mc murrichi: KWIETNIEWSKI, 1898, pp. 394-395.
Gonactinia sp.: OKADA, 1926, pp. 482-486.

, A single specimen was obtained by Mr. I. NONAKA in 1925 at Asa-
mushi.   This actinian is very well-known in Japan on account of its
swimming habit and is quite common along the Pacific coasts of Japan
from Misaki southwards, but it is probably rare in Mutsu Bay.   The
species is widely distributed in the Pacific and Indian Ocean regions,
such as the Japanese coasts including the Bonin Islands, Caroline Islands,
Thursday Island, Amboyna Island and Zanzibar in East Africa. *Boloceroides*
hermaphroditica found in Zanzibar and the Suez Canal seems to be a

hermaphrodite form of this species, because the latter is only different from the type species in sexual conditions.

Subtribe ATHENARIA
Family HALCAMPOIDAE

Peachia quinquecapitata Mc Murrich
(Textfigs. 1–4)

Peachia quinquecapitata: Mc Murrich, 1913, Proc. Zool. Soc. London, p. 963, pl. 98, figs. 1-4; Uchida, 1932, Proc. Imp. Acad., vol. 8, p. 318, fig. 1.
Peachia sp.: Uchida, 1928, Proc. Imp. Acad., vol. 4, p. 67, figs. 1-2.
Bicidium aequoreae: Mc Murrich, 1913, Proc. Zool. Soc. London, p. 967, pl. 98, figs. 5–7.

The adult actinian is commonly found burrowing in sand with no formation of any shell-like substance round it, but the young is ecto-parasitic on some Hydromedusae, such as *Aequorea coerulescens* and *Catablema multicirrata.*

Adult. The column is cylindrical terminating in a slightly contracted physa, measuring about 35 mm long and 10 mm wide in the widest portion. The surface is smooth, but when examined under a lens, it is finely granulated. All the examples so far examined have no foreign material adhering to them. The body wall is very thin, and shows clearly 12 opaque lines of insertion of the perfect mesenteries. The tentacles are 12 in number and bilaterally arranged in a single circlet. They are short, thick, tapering at the tip but not capitate. The oral disc is not particularly wide. The actino-
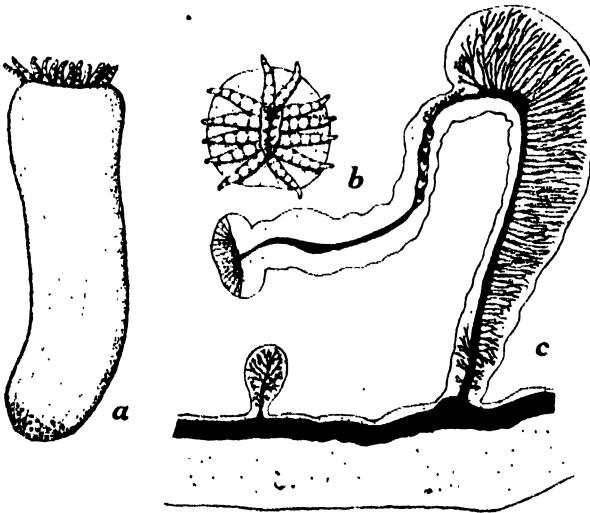
Fig. 1. *Peachia quinquecapitata* Mc Murrich; a. Side view ×3/2; b. Oral view; c. Transverse section of mesenteries.

pharynx is long and provided with a siphonoglyphe. The surface of the stomatodaeum is longitudinally grooved along the lines corresponding to the insertions of the mesenteries. The lips are roundly elevated, representing ten folds, five on each side, which correspond to the intervals between the perfect mesenteries, leaving the conchula on the line of the siphonoglyphe. The conchula is surmounted by five nipple-shaped processes which consist of two pairs lying on either side of the conchula and a larger median one situated between them on the sagittal plane of the body. The scapus is of the same width, becoming slightly narrow towards the physa. The physa is devoid of basal disc but is furnished with a central pore, round which several small papillae are radially arranged.

Anatomical aspects. The mesenteries are arranged in ten pairs, six of which are perfect, the other four being imperfect. Two of the perfect pairs are directives. Both perfect and imperfect pairs are arranged alternately in position with the exception of the median perfect one which is located between two perfect pairs.

The perfect mesenteries alone are fertile, and each is provided with a mesenterial filament. They are furnished with a well-developed long muscle band composed of numerous narrow laterally arranged branches, which again are repeatedly divided into still smaller ones. The muscle pennons are almost similar in width along the whole length, and are rather diffuse, though the axial portion is a little widened. The longitudinal pennons and the parietal muscles fuse together without distinct limits. The gonads develop in the mesenteries proximal to the muscle bands, eggs and testicular vesicles being arranged in a

Fig. 2. Diagram of transverse section through the actinopharynx of *Peachia quinquecapitata* Mc Murrich.

row in transverse sections. The imperfect mesenteries are small and almost oval in cross-section. They are each provided with an arborescent parietal muscle but destitute of mesenterial filaments. Specimens preserved in formalin generally retain a brown colour in the column and several transverse deep brown bands in each tentacle.

Young forms. The smallest young actinian obtained from the Hydromedusae is slightly larger than 1 mm in diameter. The larvae, 1–2 mm wide, are very flat and slightly convex aborally. The tentacles have not yet appeared but the peripheral margin is more or less undulating. Mouth elliptical, without siphonoglyphe, being situated in the central portion on the oral surface. There is no indication of longitudinal grooves and papillae on the side wall. In sections of these larvae, the mesogloea is thinner than the ectoderm and the endoderm, and devoid of longitudinal muscles and mesenterial filaments. The ectoderm cells are exceedingly high and full of numerous granules intensely stained by haematoxylin in their lower half, the endoderm cells being generally thickly granulated, but a few of them glandular, with a slightly widened head containing

Fig. 3. Larvae of *Peachia quinquecapitata* in the parasitic stages on the hydromedusa *Aequorea coerulescens;* a–c. Side views of actinians ×1½; d and e. Oral views of c and b respectively.

well-stained minute granules towards the inner side. Though not easily distinguished externally, eight tentacles are present, each being provided with an axial canal. In larvae, 2.5–3.5 mm in diameter, the upper margin is well-expanded, and is divided into 12 round tentacular lobes of different sizes bilaterally arranged. Along the column run eight longitudinal furrows, these indicating the lines of insertion of the mesenteries. The mouth, situated in the central portion of the oral surface, is provided with a siphonoglyphe but without a conchula. In the sections, the above-mentioned 12 tentacles, with the exception of the two directives, are arranged in five pairs. The lumens of the two tentacles adjacent to the directive tentacle which is opposite to the siphonoglyphe, are each connected with a chamber. The lumens of the two adjacent tentacles of the remaining eight open into a chamber, this resulting in the formation of two pairs of chambers. The four chambers thus formed are each provided with a short imperfect mesentery, which is to develop into a perfect one later. Mesenterial filaments are found in the old mesenteries. Longitudinal muscles are not developed in all the mesenteries. The siphonoglyphe extends deeply into the stomodacum. · The structure of the ectoderm, mesoderm and endoderm is almost similar to that described above. After these stages, the column gradually elongates aborally and becomes cone-

shaped, while the oral disc enlarges very slowly. The tentacles develop as 12 lobed marginal elongations. Along with the external changes, the internal modifications, such as the formation of the mesenteries and the development of the longitudinal pennons occur. The papillae of the conchula do not appear in the parasitic stage. The larvae in this stage exactly agree with *Bicidium aequoreae* reported by Mc MURRICH. The largest larva, found parasitic on a hydromedusa, is about 20 mm long but still has short tentacles, and the conchula is still bare. According to Dr. S. KOKUBO, the lepto-medusa *Aequorea coerulescens* is most abundant in February, and then gradually decreases in number, but becomes larger in size. About 30% of the hydro-medusae collected during May were observed to harbour 2-8 actinian larvae. These larvae were of a pale flesh colour in the living state, but some indivi-duals were slightly as bluish as the radial canals and muscles of the hydromedusa. From the similarity of coloration, Dr. KOKUBO is of the opinion that the parasites obtain nourishment from the hydromedusa. The actinian larva attaches itself to the subumbrella of the host by the oral surface, and hangs nearly perpendicularly, with the

Fig. 4. *Aequorea coerulescens* bearing two larvae of *Peachia quinquecapitata* : 2/5. Photo by Dr. KOKUBO

cone-shaped aboral end quite free. After the hydromedusa is destroyed, the actinian seems to assume a sedentary life in muddy sand. There its metamorphosis is completed and it attains sexual maturity. An account of the metamorphosis of the Indian species, *Peachia tropica* has been recently published in detail by PANIKKAR (1938).

Remarks. It may be inferred from VERRILL's description of them that the specimens, obtained from the east side of Richmond Gulf in 1899 and reported by him to be of *Bicidiopsis arctica*, are identical with the Japanese species in the number, arrangement and structure of the me-

senteries, and in the number of the nipple-like processes on the conchula, but differ in regard to the deep sulcate siphonoglyphe and the fertile imperfect mesenteries.   Several adult specimens were dredged up off Yokohama, Ôma, Ôshima, Futago and Kami-itazaki during July-August in 1926–1927.

Eloactis mazelii (JOURDAN)
(Textfigs. 5 6

Eloactis mazelii: CARLGREN, 1921, pp. 111 115, pl. 1, fig. 1; STEPHENSON, 1935, pp. 91 95, pl. 23, figs. 1 & 2.

A single specimen measuring 24 mm high and 15 mm wide in a pre-served state was obtained at a depth of about 10 fathoms off Moura by Prof. S. HÔZAWA.   This specimen is not distinctly divided into three sections.   The physa, far smaller in diameter than the oral disc, is roundly contracted and marked off from the scapus. The surface of the column, rugosely papil-lated and wrinkled, is provided with 20 dis-tinct longitudinal fur-rows, these correspond-ing to the insertions of the mesenteries. The physa, thinner in the wall than the scapus, is perforated thus having
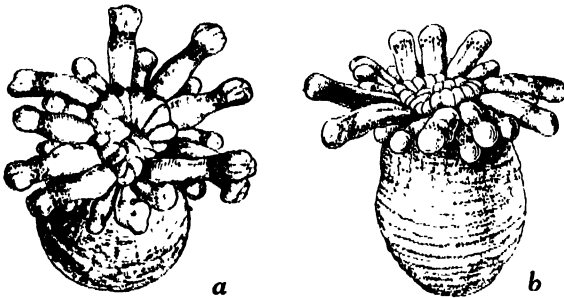
Fig. 5. *Eloactis mazelii* (JOURDAN); a. Oral view; b. Side view. × 3/2.

numerous apertures.   The oral disc is narrower in diameter than the widest part of the column, smooth and provided with radial folds, which correspond to the insertions of the mesenteries.   A well-developed ventral siphonoglyphe is present.   There is no conchula near the siphonoglyphe. The actinopharynx is marked with numerous longitudinal furrows, and covers about a quarter of the length of the body.   The tentacles are 20 in two circlets, those of the inner circlet being larger than those of the outer one.   The outer tentacles correspond to the exocoels, while the inner ones belong to the endocoels.   These tentacles are capitate and smoother than the column.   According to Mr. H. SATO, the actinian was brownish grey, with slightly bluish tentacles, which were blotched with dark brownish flecks near the rounded apical end.

Anatomical aspects. There are 10 pairs of mesenteries, two of which are directives; the ventral pair is connected with the siphonoglyphe. All the mesenteries are perfect and fertile in the adult, but the 10 pairs of mesenteries, probably corresponding to the outer small tentacles, are perfect only in the distal portion, and fertile only in the proximal portion in small individuals. The longitudinal pennons are strong and markedly circumscribed especially in the fertile region. The parietal muscles are weak. Both marginal and oral stomata are large. There is no marginal sphincter.

Remarks. This species seems to be rather common and is known to exist in the northern parts of Europe and the Mediterranean Seas, but its existence has not hitherto been recorded in the Pacific. Several specimens belonging to this species were also found in the collection from Onagawa Bay. The colour seems to be somewhat different from that of specimens found in Europe, but the European specimens are also very variable in coloration.
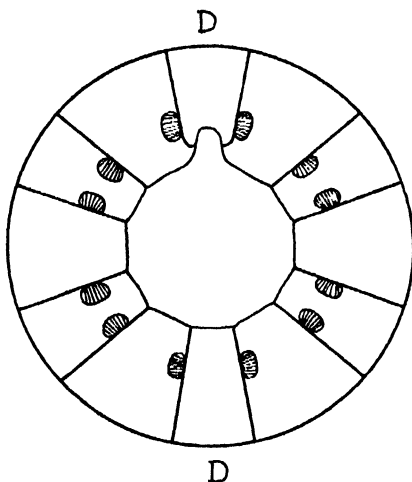
Fig. 6. Diagram of horizontal section through the actinopharynx of young *Eloactis mazelii.*

Harenactis attenuata TORREY
(Textfigs. 7-9)

Harenactis attenuata: TORREY, 1902, Proc. Washington. Acad. Sci., vol. 4, pp. 384–387, pl. 24, figs. 4, 5, textfigs. 16, 17., —; CARLGREN, 1936, ditto, vol. 26, p. 19.

A single specimen was obtained by Prof. HÔZAWA on July 24, 1926 off Kami-itazaki, and three examples by Mr. NONAKA in 1925 from the bay. They all seem to be immature specimens of the American species. The body is changeable, owing to being in a stage of contraction, cylindrical, 21 mm long and 3 mm wide, in a preserved specimen, divided into capitulum, scapus and physa. The wall of the column in the preserved specimens is intricately wrinkled transversely. The oral disc is concealed among the tentacles and long oval in shape, the mouth being situated in the central portion of the disc, with folded lips and a single siphono-
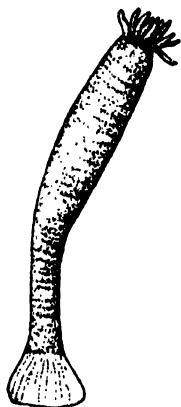
Fig. 7. Side view
of *Harenactis attenuata*
TORREY. ×2.

glyphe but no conchula. The tentacles, 24 in number, are arranged in two alternative circlets. These tentacles are nearly similar in length, rather broad and each with a bluntly pointed tip, as shown in Fig. 4, Pl. 24 by TORREY (1902). On the surface of the column can be seen the indications of 24 insertions of mesenteries. The column is widest in the middle length and narrows near the physa. The physa, though variable when contracted, is ampullaceous, thin-walled and becomes wide basally. The mesenteries are visible from outside through the wall of the physa. The colour in preserved specimens is dull reddish.

Anatomical aspects. There are 24 pairs of mesenteries grouped in two series; the first series is nearly perfect along the part corresponding to the actinopharynx, while the second is imperfect with the exception of the upper short limited part. The mesenteries of the first series are provided with partial muscles, muscle pennons and mesenterial filaments. The gonads had not yet developed in any of the mesenteries. The parietal
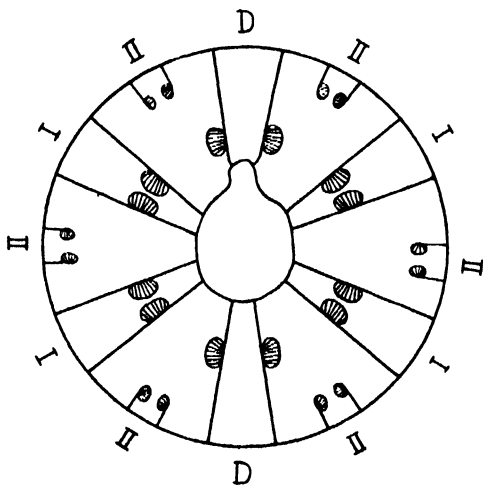
Fig. 8. Diagram of horizontal section through actinopharynx of young *Harenactis attenuata*.

Fig. 9. Section of a directive mesentery of young *Harenactis attenuata*.

muscles and longitudinal muscle pennons are composed of about 15 or more branches, which are again divided into several branchets. The muscle pennons are most complicated in the parts with the mesenterial filaments, but not so much as shown in TORREY's figures, because the Japanese specimens are all in the earlier stage of growth. The muscle pennons are in the intermediate stage between the circumscribed and diffused states. The mesenteries of the second series are provided with parietal muscles only, which are slightly more weakly developed than in the mesenteries of the first series, and have short mesenterial filaments.

Remarks. The species was found in San Pedro and Newport, both in California. The Japanese specimens, which are smaller in size and provided with mesenteries which are less differentiated, are probably immature examples of this species.

Family ANDWAKIIDAE

Andwakia hozawai UCHIDA
(Textfigs. 10-12)

Andwakia hozawai: UCHIDA, 1932, Proc. Imp. Acad., vol. 8, pp. 394-396.

Sixteen specimens were collected by Prof. HôZAWA on July 1926 at Asadokoro in Asamushi Bay. The actinian occurs buried in sandy bottoms, probably without any covering, being permeated with detritus particles. The surface of the column wall is hard and papillated. The column is thin, hard and leather-like, while the mesogloea is universally thin. The body, though extremely variable in form owing to contraction, is broadly cylindrical and somewhat cornucopia-like. A well-preserved specimen is 55 mm long, and 10 mm wide in the widest distal part and 4 mm in the narrowest proximal part of the scapus. The capitulum is short and liable to be withdrawn into the scapus, which is wider than the former. When slightly contracted, the capitulum is clearly marked off from the scapus by a collar-like fosse. The tentacles are about 60-80 in number, perforated at the tip, tapering, filamentous, and arranged in 5-6 cycles, the outer tentacles being slightly smaller than the inner ones. The oral disc is rather small,

Fig. 10. *Andwakia hozawai* UCHIDA; Side view. ×1.

with a slit-like mouth in the central portion. The siphonoglyphes are two in number, and the lips are somewhat elevated, forming 13–15 fold-ings. The scapus is elongated, and can be divided into two parts; the comparatively narrow proximal part is about 1/3 the length of the scapus, while the broader distal part is widest in the middle portion. The surface of the scapus is generally smooth, and seems to be devoid of conspicuous papilla-like excrescences. The insertions of the mesenteries in the scapus and in the physa are obvious from the outside. A few acontia are often observed to be emitted from the surface of the body-wall through cinclids distributed in the middle portion of the scapus. The physa is ampullaceous, and very conspicuous in well-preserved specimens, but is often reduced to an enlargement merely, in contracted specimens. The colour of specimens preserved in formalin is bluish grey.

Anatomical aspects. The tentacles consist of a very thick mesogloea furnished with ectodermal ring muscles, which give rise to many centri-fugal processes, and of thin endoderm. Around the marginal portion of the capitulum the mesogloeal sphincter feebly develops. The ectoderm of the scapus is generally thicker than the mesogloea and the endo-derm, though the latter two become quite thick near the basal portion. The inner layer of the actinopharynx is highly folded and made of high cylindrical ectoderm cells, containing many gland cells. The mesogloea has many centrifugal cores, one in each fold. The mesen-teries are arranged in 24 pairs, of which six are per-fect and 18 imperfect. Of the six perfect pairs two
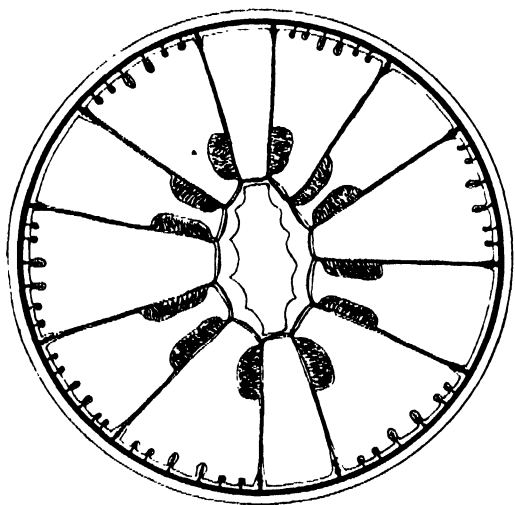
Fig. 11. Diagram of horizontal section through the actinopharynx of *Andwakia hozawai.*

are the directives. The perfect mesenteries are supplied with a parietal muscle and well-developed longitudinal muscle pennons situated proximally to the actinopharynx, which is connected with the endoderm walls of the coelom by means of two terminal lamellar parts. The cross-section of

the muscle pennons is generally circumscribed, but circumscribed-diffused below the actinopharynx, and always provided with a number of parallel muscle processes. The mesen-
terial filaments, generally three-
lobed at the tip, are absent in
the mesenteries near the basal
portion. The imperfect mesen-
teries are divided into two
groups, six pairs of the secon-
dary and 12 pairs of the third
cycle. These mesenteries are
destitute of mesenterial fila-
ments, and are furnished with
a parietal muscle, having seve-
ral processes on both sides.
The gonads develop only in
the perfect mesenteries, the
ova or testicular vesicles being
arranged in a row in the long
tract of the mesogloea. The
acontia, present in the middle
and rather lower portions of
the scapus, are few in number.
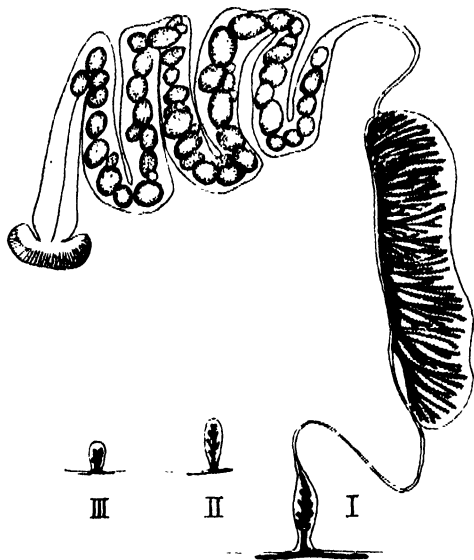
Fig. 12. Sections of mesenteries of the first, second and third series of *Andwakia hozawai*.

Remarks. The genus *Andwakia* has hitherto been represented by the single species, *Andwakia mirabilis* DANIELSSEN, which was found in Husöen, Sognefjord, on the western coast of Norway. On account of the disparity in the number of tentacles, the presence of imperfect mesen-
teries of the third cycle and the absence of the covering round the column, the Japanese actinian seems to be quite different from the Norwegian species.

Subtribe ENDOMYARIA

Family BUNODACTIIDAE

Anthopleura stella (VERRILL)

(Pl. XI, Fig. 4; textfigs. 13-16)

Bunodes stella: VERRILL, 1864, pp. 16-17, pl. 1, figs. 1-8.
Bunodactis stella: VERRILL, 1899, p. 43.
Cribrina stella: MC MURRICH, 1910, pp. 76-77, pl. 3, figs. 6-7; CARLGREN, 1921, pp. 148-151.

Tealinopsis stella: VERRILL, 1922, pp. 112 G -113 G, pl. 20, figs. 4 -12; pl. 26, figs. 1 -6 & pl. 31, fig. 3.

Cribrina artemisia: ASANO, 1911, pp. 138-139, pl. 2, fig. 11.

? Cribrina elegantissima: MC MURRICH, 1901, pp. 18-23, pl. 1, fig. 7 & pl. 2, figs. 8-14 (red coloured variety ?)

? Cribrina sp.: WASSILIEFF, 1908, p. 18, Taf. 1, Fig. 1; Taf. 5, Fig. 59 & Taf. 6, Fig. 60.

Since this species has been generally described on the basis of preserved and contracted specimens, the marginal sphaerules have hitherto been overlooked. In VERRILL's previous paper (1864) are given figures of expanded specimens drawn by E. S. MORSE (Pl. 1, figs. 5 & 7). The actinians illustrated there have distinct marginal swellings probably corresponding to marginal sphaerules. In Japan, this actinian has long been wrongly named *C. artemisia*. As to the living state and the coloration of this species VERRILL (1864) gave the best description, and, as regards the internal anatomy, MC MURRICH (1910) went fully into the details.

When fully expanded, the actinian is pillar-shaped with the middle portion narrowest, enlarging more rapidly towards the disc than towards the base. When elongated, the column is long and cylindrical, its height being more than twice the diameter. Ordinarily, the height is only slightly larger than the diameter, measuring generally 20–60 mm high and 15–40 mm wide. When contracted, the column becomes dome-like, the upper part being thickly covered with verruciform suckers, which are sparsely distributed but arranged in regular vertical rows in the lower part. The tentacles are broad and about equal in length to the diameter of disc, being always regularly arranged in the following five cycles, 6, 6, 12, 24, 48. They are each wider near the base, and gradually taper to the obtuse tip. Those of the first two cycles are slightly larger than the rest, forming the inner row of twelve, which are generally held in an upright position during expansion, while the others are mostly curved more or less outwards and, especially those of the fifth cycle, downwards. The tentacles of the first, second
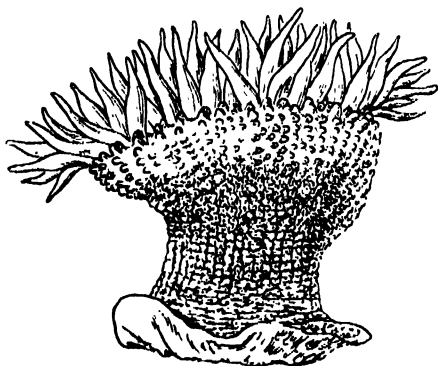
Fig. 13. Side view of a preserved specimen of *Anthopleura stella* (VERRILL). ×1.

and third series may extend beyond the diameter of the expanded oral
disc, but the tentacles of the fifth cycle are always shorter than the oral
disc. Round the margin of the disc, just below the fifth cycle the ten-
tacles often stand round marginal sphaerules in a sparse row. The mouth
slit-like, often with elevated lips on the directive line, is situated in the
centre of the oral disc. The column with the exception of the bases, is
covered with verruciform suckers which are arranged in about 48 vertical
rows, more thickly set in the upper part, but very sparsely near the base.
These suckers in the living specimens are papillated but transversely
elliptical, with a central cavity and a raised crenulated margin in the
preserved specimens. In contracted specimens, the suckers are often so
crowded near the marginal portion as to be nearly in contact, being only
separated by wrinkles. The suckers situated nearer the margin in a
vertical series are generally larger. In its habitat, the actinian has peb-
bles and fragments of shells adhering to its suckers, but when brought
into an aquarium it discards them in a few hours. When the actinian
contracts in an aquarium the suckers are observed to eject water.

The coloration of the oral disc and of the column is variable to some
extent, but that of the verruciform suckers is always greenish. The oral
disc, varies in colour, being brownish green or greenish brown, generally
dark reddish brown near the bases of tentacles and greenish round the
mouth. From the bases of the primary tentacles outwards radiate pairs
of white bands to the margin of the oral disc, the lips of these disc often
being tinted bright green, the stomodaeum white. The tentacles are
white, slightly brownish or slightly pinkish (especially in specimens from
the warmer parts of Japan). When contracted they are yellow or pink.
In large specimens the shaft of the tentacles is mottled with white spots,
their bases being generally white on their abaxial side. The marginal
sphaerules are whitish brown. The column is generally greenish black,
with green verruciform suckers. Near its base the column is often flesh-
coloured or yellowish grey. It is noticeable that some specimens collected
at Oshoro have a reddish column wall with green verruciform suckers.
These specimens resemble in colour *Cribrina elegantissima* reported by
Mc Murrich to exist in Puget Sound.

Anatomical aspects. The ectoderm cells are high, cylindrical and
vesiculated, the endoderm cells being low cylindrical, laden with black
granules in the upper half, and vesiculated in the lower half. The ecto-
derm cells of the tentacles are very narrow and chain-shaped, and their
endoderm cells are mostly granulated and glandular. The muscles of

tentacles are ectodermal and sparsely branched. The outer surface of
the marginal sphaerules is largely furnished with long spirocysts. The
sphincter is well-developed, nearly kidney-shaped, circumscribed pedunculate,
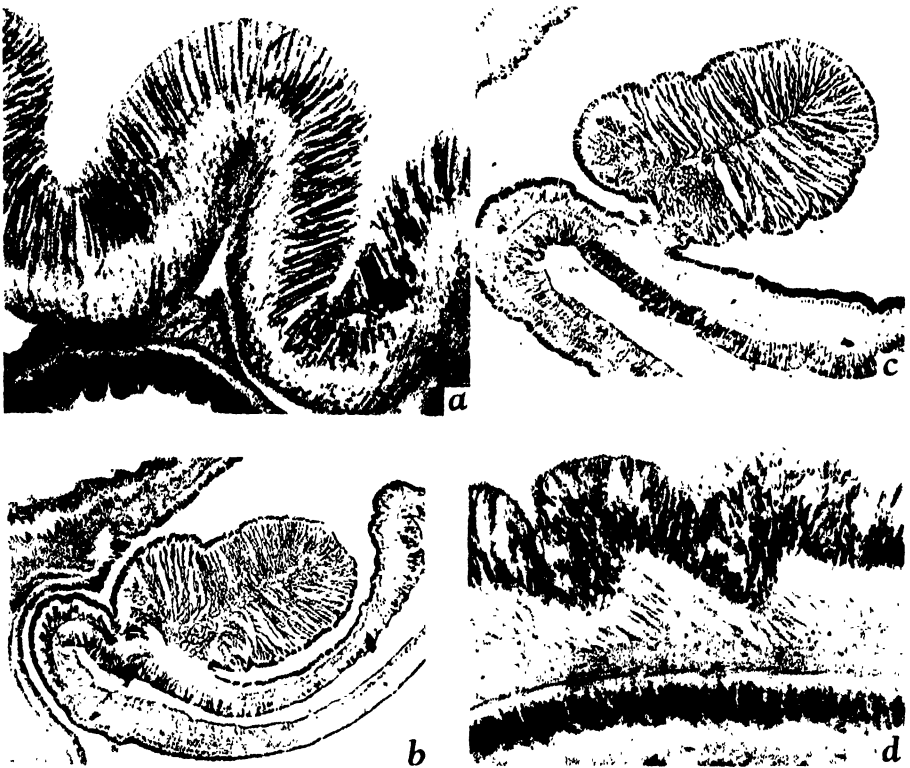
Fig. 14.   *Anthopleura stella* VERRILL.; a. Sagittal section of marginal sphaerule,
×180; b c. Radial sections of a sphincter in two different parts, × 40; d. Transverse
section of a part of tentacle. ×180.

with numerous anastomosed foldings. The siphonoglyphes are two in
number. The mesenteries are hexamerously arranged; in the four cycles,
6, 6, 12, 24. The first two cycles of the mesenteries are perfect, con-
taining two directives. With the exception of the directives, all the
mesenteries are provided with mesenterial filaments and are fertile. The
longitudinal muscles of these mesenteries are well-developed, and diffused
circumscribed; the muscle pennons of the directives are grouped in several
foldings; those of the first and the second mesenteries are narrow in
width but extend for a fair length showing shallow foldings; the muscles

of the third mesenteries are rather circumscribed, and those of the fourth mesenteries are often crescent. The parietobasilar and basilar muscles are well developed, especially in the lower part.

Distribution. The actinian is common in Mutsu Bay in shallow pools near low-water mark, buried to the tentacles in sand, occupying the cracks and crevices of ledges covered with algae. This is one of the commonest species on the coasts of Japan from Hokkaido to Kyushu. It is also known on the Atlantic and Pacific coasts of North America and further on the coasts of Northern Europe. The Japanese actinian seems to reach a larger size than that in other localities.

Remarks. The description of the external features of this species in the living state has been based entirely on the field notes of collectors and never been verified by reference to the investigators' observations with the exception of those of ASANO. The colorations hitherto described regarding this species, however, are in general agreement, though

Fig. 15. Diagram of horizontal section through the actinopharynx of *Anthopleura stella*.

Fig. 16. Sections of mesenteries of *Anthopleura stella*.

the descriptions are rather brief and not in detail. The presence of the bright green verruciform suckers seems to be one of the most important characters of this actinian. *Cribrina elegantissima* described by Mc MURRICH corresponds with the present species in the preserved state, in the section of the sphincter etc. and, furthermore, resembles in colour reddish specimens of the same species from Oshoro. But for the present the writer cannot say that they are definitely identified. ASANO who examined specimens from Misaki where this actinian occurs very abundantly, described an actinian under the name of *Cribrina artemisia*. From his description and figures, that actinian is quite different from *Cribrina artemisia = Anthopleura xanthogrammica* but is most likely to be identified with *A. stella*. The marginal sphaerules are hardly visible in preserved specimens, and, even in living examples are liable to escape the observation of zoologists other than specialists for the Actiniaria. Therefore, in the description of this species the sphaerules have not been recorded. ASANO reported that his actinian has pseudo-acrorhagi, but as shown in the figure of a section here given, they are true acrorhagi = marginal sphaerules, which are studded with clusters of nematocysts.

Anthopleura xanthogrammica (BRANDT)
(Textfigs. 17-18)

Anthopleura xanthogrammica: Mc MURRICH (probably different species), 1901, pp. 36-39, pl. 2, fig. 14 et pl. 3, figs. 21-24; TORREY, 1906, pp. 41-46, pl. 8; ASANO (probably referable to *Anthopleura japonica*), 1911, pp. 139-140, pl. 2, fig. 13; CARLGREN, 1934, pp. 349-351.

Anthopleura japonica: ASANO, 1911, p. 140, pl. 2, fig. 14.

Bunodes californica: FEWKES, 1889, pp. 28-30, pl. 6, figs. 5, 6.

Cribrina artemisia: Mc MURRICH, 1901, pp. 23-26, pl. 2, figs. 15-16; pl. 3, figs. 18 20; TORREY, 1902, p. 390, pl. 25, figs. 1-3.

Evactis artemisia: VERRILL, 1922, pp. 113 G-114 G; CARLGREN, 1934, pp. 16 17.

Anthopleura xanthogrammica and *Cribrina artemisia* have often been described as two separate species, but the main difference lies in the presence or absence of the marginal sphaerules. These two species have been mostly reported on on the basis of preserved specimens; in the preserved state, the marginal sphaerules are flattened or distorted, and liable to be readily overlooked. In 1906 TORREY having examined many living specimens came to the conclusion that the two species must be conbined in the single species, *A. xanthogrammica*.

The body is very changeable relatively to conditions, very extensile and long cylindrical when buried in sand below sea-water, but when

brought into an aquarium, it becomes lower in height. In the elongated state, it reaches 90 mm long, the distal portion being rounded and wide, while the rest is narrow and long cylindrical. When the actinian is preserved in the elongated condition, it often takes the form as illustrated by Mc MURRICH (fig. 15, pl. 2, 1901) for *Cribrina artemisia* Anthopleura xanthogrammica. Expanded specimens in aquaria are generally 30-60 mm in diameter and 30 50 mm

Fig. 17. Preserved specimens of *Anthopleura xanthogrammica* BRANDT. × 1.

high. When contracted, these specimens become cylindrical, nearly equal in width throughout the whole length, with verruciform suckers, which cluster round the oral margin and sparsely distributed lower down. The tentacles, are all shorter than the diameter of the oral disc, slenderer than those of *A. stella*, the oral side of them being in most cases traversed by irregular white flecks, which are generally about ten in number. These flecks are almost always present. The 12 tentacles of the first and the second cycles are directed axially in the well-expanded condition, while the rest are directed outwards and bent downwards. The mouth is slit-like, situated in the centre of the oral disc. The marginal sphaerules, varying in number with individuals, are found above the uppermost verruciform suckers, which are generally two- or three-lobed. The column is set with verruciform suckers which are swelled and cone-shaped in well-expanded specimens but long and papillose in preserved ones. The uppermost verrucae are the largest and alternate in position with the marginal tentacles. The verruciform suckers have each a pit in the centre. They are larger and longer nearer the oral disc, and, especially at a short distance from the oral margin, very crowded, forming "a papillose collar" in contracted specimens, but below the "papillose collar" the suckers become suddenly low and are sparsely distributed. The column holds gravels and fragments of shells attached round the oral margin, but holds none in the lower part. Near the basal portion there are no suckers. The basal disc, firmly adhesive, is smaller in diameter than the oral disc.

The colour is rather variable in the oral disc and tentacles, but nearly

similar in the column.   The tentacles are generally brownish grey with flecks, with a whitish shade on the abaxial side.   The 12 tentacles of the first and second cycles of large specimens are often slightly pinkish purple on the abaxial side.   Specimens having brownish green or green tentacles are still found.   In these the abaxial side of the tentacles is greenish white.   In one specimen the tentacles were a pinkish purple. The oral disc is nearly the same in colour as the tentacles: the grey tentacled ones are provided with the greyish oral disc, and the green tentacled with the greenish oral disc.   From the periphery of the epistoma radially issue white streaks, varying in individuals in broadness and distinctness, which seem to correspond to the number and order of the tentacles, thence forming several radial groups.   These white streaks are sometimes slightly pinkish in shade.   Both sides of the base of the tentacles of the first and second cycles are prominently marked with the broad white streaks.   The verrucae crowded near the upper portion of the column, having sand and gravel attached to them, are always greyish olive green.   The marginal sphaerules are brownish yellow.   The lower part of the column is yellowish brown or flesh-coloured.

Anatomical aspects.   Ectoderm cells generally cylindrical, with a nucleus in the middle portion, the lower part being vesiculate, with the nerve
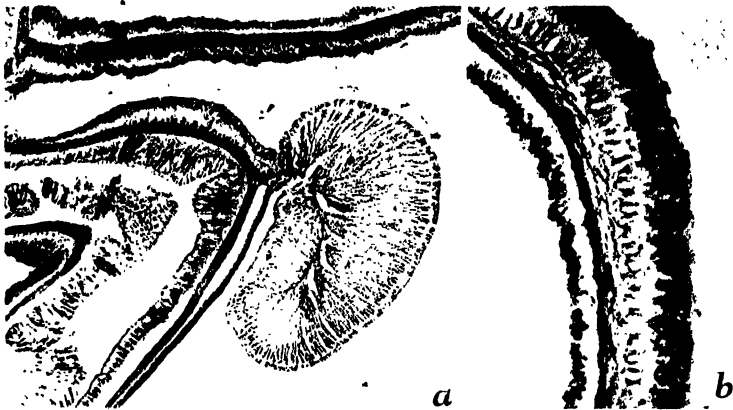
Fig. 18.   *Anthopleura xanthogrammica* BRANDT;   a. Radial section of sphincter. ×40;   b. Transverse section of tentacle. ×180.

net at the base.   Those in the column generally filled with small black granules.   Ectoderm cells in the actinopharynx, high cylindrical, glandular, vacuolate and with cilia.   Endoderm cells rather low, cylindrical, filled with black granules.   There are marginal sphaerules, of which the basal

part is vesiculate and glandular, but the larger part is closely set with long spirocysts. Muscles of the tentacles ectodermal, slightly branched. Muscles of the oral disc near the mouth, endodermal, with short arborescent foldings. Sphincter, nearly elliptical in shape, circumscribed asymmetrically pedunculate pinnate, with numerous foldings, as figured by Mc MURRICH (1901) in *Cribrina artemisia*. The mesenteries are arranged in $6+6+12+24=48$ pairs, 2 of which are directives. In the upper portion of the actinopharynx, 24 pairs are perfect but in the lower part 12. The mesenteries, except the directives, are all fertile in large specimens. The muscle pennons are well-developed. The parietobasilar and basilar muscles are strong.

Distribution. The actinian is known on the Atlantic and Pacific coasts of North America. It is distributed also in the Behring Sea (Kamchatka and Alaska). In Japan it is commonly found on the coasts of Hokkaido and the Northern parts of Honshu. It is found buried in sand, attached to the substratum, just below the tidal lines. In this condition the column is generally very elongate, with gravels and fragments of shells adhering round the oral margin. In the case of contraction after being taken out of the water the actinian spurts water from the upper verruciform suckers.

Remarks. The reason why this species has been binominally described under the name of *Anthopleura xanthogrammica* and *Cribrina artemisia* is that the two forms have been hitherto described mostly from preserved materials. In preserved specimens of this species, especially in those with the contracted oral disc, the marginal sphaerules are hardly distinguishable from contracted tentacles and verruciform suckers. Except for a brief original description, *Cribrina artemisia* has been mainly described only on the basis of preserved specimens. Having examined living and preserved specimens, the present writer is also of the same opinion as TORREY that the two actinians must be combined in the single species, *Anthopleura xanthogrammica*. This actinian is distinctly characterized by the presence of the white spots along the tentacles and of the white radial streaks on the oral disc, and also by the olive verruciform suckers thickly massed in the limited distal portion of the column and by the flesh-coloured column in which low flesh-coloured verruciform suckers are distributed. The features of the column are visible in preserved specimens. This species is easily distinguishable from *Anthopleura stella* by the characteristics above-mentioned. Moreover, this species is characterised by its changeable column, as TORREY states, "The form of the column varies accordingly, very long individuals taken from the sand

becoming much shorter than broad in aquaria." CARLGREN (1934) pointed out that the actinian reported by KOMAI and IKARI (1929) as *Anthopleura xanthogrammica* (the identification is probably due to ASANO's original paper, 1911) may possibly be *Anthopleura japonica* briefly mentioned by VERRILL (1899). The writer, though somewhat doubtful about the identification, distinguished here *A. xanthogrammica* from this actinian which is distributed in the southern parts of Japan, and seems to be identified with VERRILL's *A. japonica*.

Anthopleura japonica VERRILL
(Pl. XI, Fig. 3; textfigs. 19-21)

Anthopleura japonica: VERRILL, 1899, p. 218; ASANO, 1911, 140, pl. 2, fig. 14 (=*A. xanthogrammica*).
Anthopleura xanthogrammica: ASANO, 1911, pp. 139-140, pl. 2, fig. 13.
Anthopleura mc murrichi: WASSILIEFF, 1908, pp. 19-20, Taf. 1, Fig. 14; Taf. 6, Figs. 61-64; Textfig. 6.

This actinian is common on the southern coasts of Honshu and Kyûshu. The separation of this species from *Anthopleura xanthogrammica* remains for future investigation but, following CARLGREN (1934), the actinian is

Fig. 19. Preserved specimen of *Anthopleura japonica* VERRILL. ×1.

described under the name. Body changeable, sometimes long rather than wide, sometimes wide rather than long, generally 4-7 mm high and 4-6 mm wide in the widest diameter. The oral disc expands more widely than the pedal disc, the middle part of the column being the smallest in diameter. Tentacles rather short, long cone-shaped, arranged in 5 cycles, 6, 6, 12, 24, 48, almost similar in length in large specimens, but in general the 12 tentacles of the first two cycles are slightly larger than the rest and extend upright, with rapidly narrowing prehensile tips. Just outside the tentacles are arranged marginal sphaerules in a row. Column widest in the upper portion and narrowest in the middle length; the upper portion below the oral disc is thickly furnished with many adhesive suckers, which become large and cone-shaped in their expanded condition. The uppermost suckers are more or less lobulated. The adhesive suckers are smaller and more sparsely

distributed in the lower half. The actinian has pebbles and shells adhering to the suckers and ejects water from these when it contracts.

Colour not very variable. Oral disc bluish brown or deep brown or sometimes with radial stripes. Epistome brownish purple or deep brown. Lips with a few white flecks. Tentacles dark brown, sometimes reddish brown, especially in the slightly contracted state. Generally no white flecks as in *A. xanthogrammica*. Marginal sphaerules white or yellowish white. Column yellowish brown in young individuals but dark brown in large specimens. Verrucae same in colour as the column. Some individuals dark bluish in the column, and some dark brown in the upper column and dark bluish in the lower part.

Anatomical aspects. Ectoderm high-cylindrical, containing many glandular cells among supporting cells. Endoderm high-cylindrical, glandular, the lower half generally vesiculate and containing *Zooxanthellae*, which
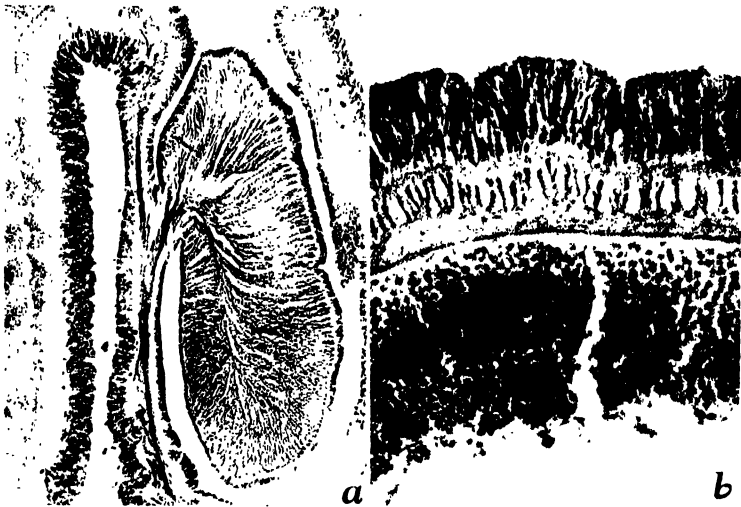
Fig. 20. *Anthopleura japonica* VERRILL; a. Radial section of sphincter. ×40; b. Transverse section of tentacle. ×180. The endoderm of the tentacle contains large number of symbiotic algae.

are especially numerous in the tentacular endoderm. Tentacles with well-developed ectodermal muscles, numerous nematocysts in the ectoderm and many symbiotic algae in the endoderm. Marginal sphaerules thickly studded with nematocysts. Siphonoglyphes two in number. Sphincter, well-developed, elongate elliptical, circumscribed, pedunculate pinnate, with numerous foldings which are complicately anastomosed. Mesenteries

hexamerously arranged, $6+6+12+24=48$, including two pairs of direc-
tives, those of the first and the second are perfect and large, while those
of the third and the fourth are imperfect and small. All the mesenteries are provided with mesenterial filaments and fertile with the exception of the direc- tives. Muscle pennons are well- developed but rather diffused and narrow in width in all these mesenteries. The muscle pennons of the first mesenteries extend farthest along the mesenteries, and those of the second mesen- teries are slightly shorter than these. The muscle pennons of the third mesenteries are shorter than the former two, but rela- tively wider than them. Those of the fourth mesenteries are smallest and often irregularly shaped.
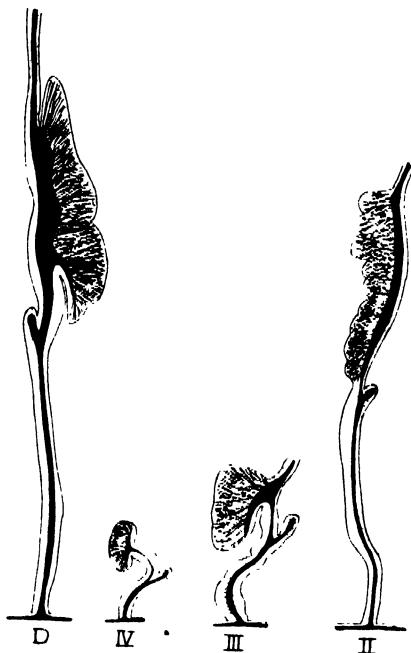
Fig. 21. Sections of mesenteries of *Antho-pleura japonica* VERRILL.

Distribution. This actinian is common on the coasts of Honshu and Kyushu. It lives on the tidal line on elevated rocks above the water in ebb-tides and is generally covered with pebbles and shells attached to the verruciform suckers. Actinians probably referable to this species were reported as found in Japan from Shimoda, Izu Prov. by VERRILL and Enoshima by WASSILIEFF.

Remarks. The species is seemingly distinguishable from *A. xantha-grammica* by its non-spotted reddish brown tentacles and by the column, which is brown in colour. The verrucae are distributed in the more lower part and do not present such conspicuous elongations as the latter. The endoderm cells contain numerous symbiotic algae, which have not been found in Japanese specimens of *A. xanthogrammica* living in the same locality as this species. However, it is probable that the difference in the coloration and habitats of the two species are correlated with the presence or absence of the symbiotic algae. On the other hand, TORREY

(1906) reported in the case of for *A. xanthogrammica* that "the characteristic green color of the species is found only in individuals exposed to the sun. It is due to the presence of a unicellular alga in the endoderm of the column wall, mesenteries and tentacles. Where sunlight does not penetrate, as under wharves (CALKINS), or in caves, the algae, though present, do not develop so luxuriantly as in more exposed situations, and the polyps are correspondingly pale." Specimens with a green oral disc and tentacles are often observed in Japanese specimens of *A. xanthogrammica*, but in *A. japonica* the oral disc is always bluish brown or brown and the tentacles brown or reddish brown. Moreover, this species does not occur in the northern parts of Hokkaido. To determine the identity of these two species the histological investigation must be further gone into. The actinian described by WASSILIEFF as *A. mc murrichi* n. sp., based on a single specimen obtained at Enoshima, seems to be identical with this species, because the external features in the preserved condition (Taf. 1, Fig. 14) and anatomical characters generally agree with those of the present species, though the figure of the sphincter of WASSILIEFF's specimen is too vaguely drawn. The original description by VERRILL of this species is restricted to the external features of a preserved specimen from Shimoda. In the same locality *A. japonica* occurs in abundance.

Anthopleura pacifica n. sp.
(Pl. XI, Fig. 2; textfigs. 22-23)

Anthopleura xanthogrammica: Mc MURRICH, 1901, pp. 36-39, pl. 2, fig. 17; pl. 3, figs. 21-24 (in part).

Among Japanese species belonging to *Anthopleura*, this actinian is characterized by its small size and red coloration. Body generally 15–20 mm high and 12–18 mm wide, but rarely more than 30 mm high and 25 mm wide in large specimens. Body variable in form in aquaria, becoming very elongated, and forming a long cylindrical shape, with the expanded distal part. Tentacles about 60–80 in number, starting generally from near the margin of the oral disc, though somewhat hexamerously, not regularly arranged probably on account of asexual reproduction. The tentacles are thick and firm, rather short, always shorter than the column-height and not filamentous. Oral disc, wider than the column, flat and with a slit-like mouth in the centre. Marginal sphaerules about 20 in number, round and arranged in a circlet. Column with verruciform suckers, which are rather indistinct and arranged in about 48 rows. The suckers

are larger near the marginal portion of the column, and are not found in the basal portion. Being sometimes very small, most of them may, with the exception of the premarginal ones, be overlooked in a careless observation.
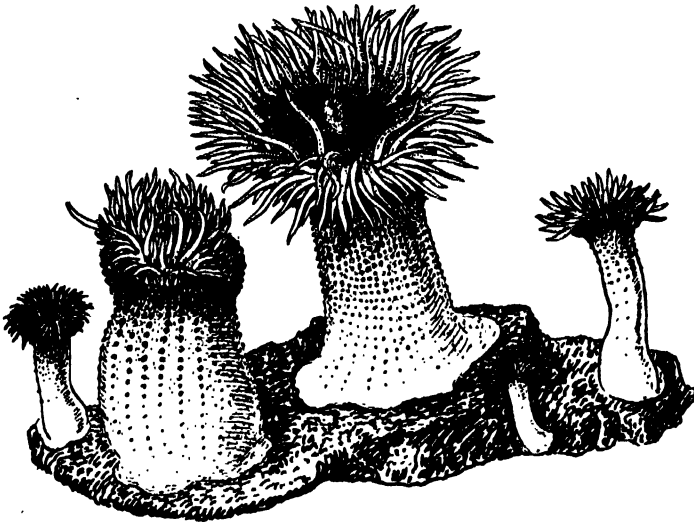
Fig. 22. *Anthopleura pacifica* n. sp. ×1.

Colour almost constant. Column pinkish red in the lower portion, but greenish in the upper, especially near the oral disc. Verrucae green in the central portion. Marginal sphaerules pale pink. Tentacles slightly pinkish on the axial surface and slightly greenish on the abaxial surface. They are spotted with several transversal white patterns. Oral disc mainly dark red with a green nuance, with some radial pinkish red patterns. Lips pinkish.

Anatomical aspects. Ectoderm very high, seemingly composed of two layers of cells; the outer cells being mostly glandular and eosinophile, the inner cells narrow and connected with nerve cells at their bases. Mesogloea thinner than the ectoderm, containing wandering cells sparsely distributed and giving rise to minute processes of the endodermal muscles. Endoderm composed of conical finely granulated glandular cells which are laden with many dark granules only in the upper portion. The ectoderm cells of the basal disc are narrow cylindrical and glandular, the outer surface of the ectoderm being bordered with widened heads of narrow gland cells; the basal portion contains many long eosinophile

glandular parts. The marginal sphaerules with the exception of the basal portion, are covered with slender nematocysts, which are thickly set in a

Fig. 23. *Anthopleura pacifica* n. sp.   a. Transverse section of tentacle. ×180; b d. Radial sections of sphincters of different specimens. ×40; e. Siphonoglyphe supported by a pair of non-directive mesenteries as the result of asexual multiplication. ×76.

row. The ectoderm of the tentacles is about four times the height of the endoderm. The ectoderm cells are chain-shaped, covered with long spirocysts and connected with the nerve nets at their bases. The tentacle muscle are ectodermal and slightly folded. The mesogloea of the tentacles is thin. The endoderm of the tentacles contains thick plasm, laden with granules well stained by Haematoxylin and pigmented granules. The

sphincter is generally small, round or elongated, circumscribed pedunculate palmate but pinnate in large specimens. There are more than two siphonoglyphes on account of asexual fission. They are frequently supported by a pair of large mesenteries, which are not directives. These siphonoglyphes are surmised to have secondarily formed in the parts corresponding to the mesenterial parts of the first series after the fission. When the fission is repeated, the actinian comes to have more than two siphonoglyphes. In fact, the histological differentiation of the siphonoglyphes in one actinian is more or less variable. The mesenteries especially in young specimens seem to have the tendency to be arranged as $6 + 6 + 12 + 24$, but probably on account of frequency of the fission, the arrangement is generally in disorder. The directive mesenterial pair could not be in most cases observed, and the partner of the mesenteries only stands here and there between the mesenterial pairs. The first two series seem to be perfect, but the third and fourth are imperfect. The mesenteries have well-developed longitudinal muscle pennons, which are diffused-circumscribed in the first mesenteries and long diffused in the other ones. Parietobasilar muscles well-developed in the lower portion. Gonads and mesenterial filaments develop on the first, the second and the third series of mesenteries.

Distribution. The actinian is commonly found in a colony on rocks and between· cracks and in shallow pools of sea-water at low tide. The species is at present known to exist in the southern parts of Hokkaido, Mutsu Bay and southern parts of Korea. It is probably distributed on the coast of Puget Sound on the Pacific coast of North America.

Asexual reproduction. While examining the mesenteries of this species it was found that their arrangement generally seemed to be regular, but that in two ·or more places, especially between the directives, a mesenterial pair lacked a partner, and, therefore, the arrangement was in disorder, often giving rise to a few small mesenterial pairs there. When the actinians were reared in aquaria, some small individuals firmly attached to the substratum, became flattened and elongated in two opposite directions, with the pedal disc being gradually divided into two equal parts.

Remarks. This species resembles *Bunodactis verrucosa* in colour and general apearance, but differs from it in the possession of marginal sphaerules and less distinct verrucae. In the comparatively small size this species is somewhat similar to *Anthopleura thallia*[1], but is different

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[1] An actinian probably referable to this European species occurs at Oshoro, Hokkaido.

from it in coloration and in the presence of more indistinct verrucae. Mc MURRICH (1901) described *Anthopleura xanthogrammica* from Puget Sound. With reference to his description CARLGREN (1934) pointed out that "the description of Mc MURRICH (1901) for *Anthopleura xanthogrammica* partially were based on other species." The actinian illustrated by Mc MURRICH in pl. 2, fig. 17 seems to be rather small, having small numbers of tentacles which rise only from the marginal portion of the oral disc, as is a characteristic of *A. pacifica* n. sp. Besides the similarity of Mc MURRICH's actinian to this new species in the small size and the form of the sphincter, the multiplication by fission is rather common in these two actinians. As to the multiplication of Mc MURRICH's specimens, he says "Dr. CALKINS states that evidences of multiplication by fission were not unfrequent among the Port Townsend specimens." On the other hand the multiplication by fission has hitherto never been observed in *Anthopleura xanthogrammica* and *Cribrina artemisia*. Though the coloration may be somewhat different, it is highly probable that Mc MURRICH's specimens belong to the species in question.

Epiactis prolifera VERRILL
(Pl. XI, Figs. 1 & 6; textfig. 24)

Epiactis prolifera: Mc MURRICH, 1901, pp. 39-43; TORREY, 1902, pp. 392 393, pl. 25, figs. 4, 5; UCHIDA, 1934, pp. 17 31, pl. 3.
Epiactis ritteri: TORREY, 1902, pp. 393-394, pl. 25, figs. 6, 7.
Bunodes japonica: VERRILL, 1869, p. 28.

This actinian is very common in Ôshima of Mutsu Bay. As stated in the present writer's previous paper, its coloration is very variable, and the form of the column also varies according to its living conditions. In the well-expanded state many white radial striations are generally observed round the mouth, the number corresponding to that of the tentacles. The column wall of this actinian is sometimes nearly smooth but sometimes prominently papillated. The papillation is different in degree, irregular in arrangement and always occurs only in the middle part of the column. This condition is quite different

Fig. 24. *Epiactis prolifera* VERRILL; small specimen. ×1.

from the papillation of other actinians belonging to the Bunodactiidae, whose columns have papillae=verrucae arranged in distinct longitudinal rows, with the larger ones near the marginal portion. This species carries

its embryos and young actinians on the column wall.

Distribution. This species is known to exist on the Pacific coasts of North America, from Alaska to California. In Japan it is found in the Kurile Islands, Hokkaido and the northern parts of Honshu southwards to Misaki.

<div align="center">

Subtribe INERMIA

Family PARACTIIDAE

Neophellia n. g.

</div>

Paractiidae with definite base. Column divided into the short scapulus and the main part; the scapulus free from sand but the main part of the column walled with a thick glandular epithelium to which sand grains adhere. Lower part of column thin-walled and lacking sand grains. Tentacles simple, in more than two cycles, their longitudinal musculature being ectodermal. Oral disc simple. Mesogloeal sphincter well-developed. Mesenteries not divided into macrocnemes and microcnemes. There are twelve pairs of fully developed mesenteries, perfect, having gonads and mesenterial filaments. The mesenteries of the first series are all furnished with diffused-circumscribed retractors. In the mesenteries of the second series inequality always occurs in the same pair; one partner is furnished with the circumscribed retractors but the other is deficient in retractors. There are another twelve pairs which are imperfect, having gonads, mesenterial filaments, but lacking retractors. All the mesenteries are fertile. Acontia are non-existent.

The genus resembles *Phellia*, *Paraphellia* etc. in general external appearance, but differs from them in having 12 perfect mesenteries and lacking acontia. The genus *Flosmaris* which alone forms the separate family Flosmarisidae resembles the new genus in the number of perfect mesenteries and in several external features, but is distinguishable in the possession of acontia and mesenteries, which are sharply divided into macrocnemes and microcnemes. On account of the absence of acontia and the inequality of the second mesenteries the new genus has been classed with the Paractiidae. In the latter the genus coincides with *Hormosa*, *Alloactis* and *Tealidium* in having mesenteries which are all fertile, but differs in the structure of the column and in the inequality of the second set of mesenteries. With regard to the inequality in the number of the mesenteries, the new genus is rather closely related to *Actinostola*.

Neophellia mutsuensis n. g. et n. sp.

(Textfigs. 25-28)

A single specimen was obtained on July 30, 1926 off Urata between Futago and Ôshima. Body in the preserved state rather cone-shaped, with rapidly expanded pedal portion and cylindrical upper portion, with the narrowest part in the middle length. Measurements as follows: pedal disc 12 mm wide, oral disc 6 mm wide, column 16 mm long, scapulus 3 mm long. Tentacles nearly 100 in number, all more or less contracted and bluntly tapering to the tips, the outer tentacles being smaller than the inner ones. The surface

Fig. 25. *Neophellia mutsuensis* n. g. et n. sp. ×2.

of the column is corrugated probably on account of shrinkage, generally permeated with sand, with the exception of the scapulus and the basal part, 3 mm long, near the pedal disc. Here, the surface is rather smooth and thin, showing indications of mesenteries. In the main part of the column are found shallow longitudinal grooves corresponding to the mesenteries obscurely visible on account of the rugose surface and the attached sand.

Anatomical aspects. Ectoderm cells of the main part of the column, high and glandular, thick with granules and protoplasm, rarely vacuolate. Mesogloea thick, rigid and fibrous. Endoderm cells glandular and vacuolate. The ectoderm of the tentacles is thickly set with nematocysts

Fig. 26. *Neophellia mutsuensis* n. g. et n. sp.; Radial section of marginal portion containing mesogloeal sphincter. ×60.

on the outer surface and with granulated cells in the inner layer. The endoderm of the tentacles is vacuolate. Muscles, ectodermal, slightly folded. Mesogloeal sphincter well-developed, with many muscular bundles which are distributed in several rows. Mesenteries arranged as $6+6+12=24$. The mesenteries of the first series are always perfect and with longitudinal
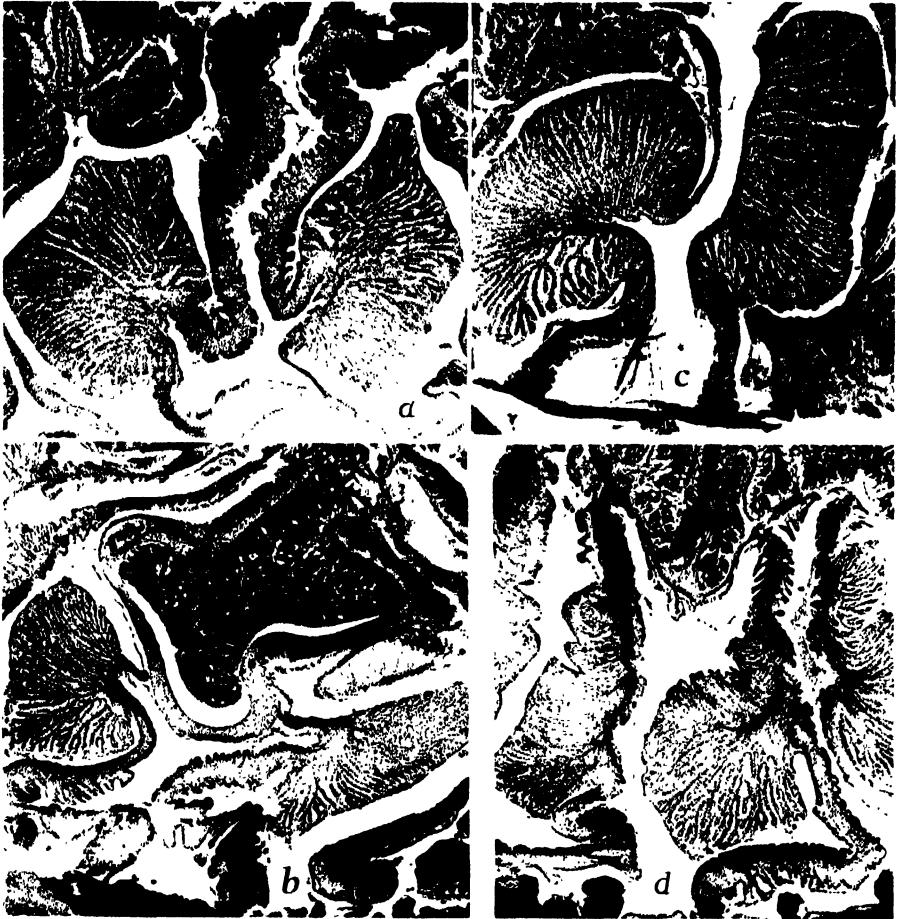
Fig. 27. *Neophellia mutsuensis* n. g. et n. sp.; a. Transverse section of directive mesenteries through the actinopharynx. ×40; b. Transverse section of directive mesenteries through the upper part of actinopharynx. ×40; c. Transverse section of directive mesenteries through the stomach. ×30; d. Transverse section of non-directive mesenteries; the middle right one being a developed partner of the second mesenteries, the rudimental partner being seen at the base to the left side of the developed partner. ×40.

muscle pennons, which are well-developed in the upper part of the stomach. The mesenteries of the second series are perfect in the upper portion but the partner of the single pair always lacks longitudinal muscle pennons. In the lower part of the actinopharynx only the partners without muscle pennons are imperfect. In the stomach the mesenteries of the second series are all deficient in longitudinal muscle pennons but bear well-developed parietobasilar muscles. The mensenteries of the third series are all imperfect, and lack mesenterial filaments. The muscle pennons of the mesenteries of the first and the second series are circumscribed, nearly triangular or round at the level of the actino-pharynx but larger and somewhat crescent in cross-section near the basal por-tion, the mesenteries of the first series being larger than those of the second. Parieto-basilar muscles present in all the mesenteries and better developed towards

Fig. 28. *Neophellia mutsuensis* n. g. et n. sp.; diagram of horizontal section through the actino-pharynx.

the basal part. Gonads develop on the mesenteries of the first and the second series with the exception of the directives.

<center>

Subtribe ACONTIARIA

Family DIADUMENIDAE

Diadumene Luciae (Verrill)

</center>

Diadumene Luciae: UCHIDA, 1932, pp. 69–82, pl. 4; —, 1936, pp. 896–906.

This species is widely distributed on the European and North American coasts, and is known as the most interesting actinian on account of the mode of dispersal. Several small specimens were collected by the present writer in Mutsu Bay. In Japan the actinian had long been wrongly named *Sagartia leucolena*.

· *Distribution.* Northern coasts of Europe, the Mediterranean Sea, Atlantic and Pacific coasts of North America. In Japan, common round the coasts of Hokkaido, Honshu, Kyushu, Shikoku (probably) and Corea. This is a shallow water form, and is generally found with pebbles and molluscan shells adhering to it.

Family METRIDIIDAE

Metridium sensile var. fimbriatum Verrill
(Pl. XI, Fig. 5; textfigs. 29-30)

Metridium sensile var. *fimbriatum:* Carlgren, 1934, p. 353.
Metridium sensile: Stephenson, 1935, pp. 214-232, pls. 15, 16, 25, 26, 27 (in part).
Metridium dianthus: Mc Murrich, 1901, pp. 3-13; Torrey, 1902, pp. 395-406; Wassilieff, 1908, p. 35; Verrill, 1922, pp. 90-92; Carlgren, 1933, pp. 22-24 (in part).

In general appearance the Pacific form *fimbriatum* of *M. sensile* cannot be distinguished from the Atlantic form *M. sensile* var. *dianthus* which is widely distributed in the Northern Atlantic coasts of Europe and North America. The Pacific *fimbriatum* is therefore generally identified with the Atlantic *dianthus* by several authorities, Mc Murrich, Torrey, Wassilieff, Verrill. Recently Carlgren (1933, 1934) studying the comparative anatomy of these two forms found that the sizes of the nematocyst capsules are considerably larger in the Pacific form than in the Atlantic, and, thence separated them into two varieties. Stephenson (1935), following him, described four varieties of this species. In Mutsu Bay the actinian is common on the shallow water coasts of Ôshima, generally forming colonies. between crevices and under rocks. Body variable in shape, sometimes more high than wide, with the voluminous disc and well-expanded base, which is firmly adherent and wider than the column. Column very extensive and variable in form, sometimes long cylindrical and sometimes short dome-shaped, widening below to the base and sharply above to the capitulum, which is marked off from the column by a distinct parapet. The surface of the column is smooth, perforated for emitting acontia. Cinclids

Fig. 29. Preserved specimen of *Metridium sensile* var. *fimbriatum* Verrill. × 3/2.

inconspicuous to the naked eye, scattered in arrangement. Capitulum, trumpet-shaped, bearing a large disc. The margin of the disc is nearly round in young specimens but generally divided into five lobes in large ones; these lobes being different in size and some of them occasionally showing a tendency to further subdivision in large actinians. Tentacles numerous, growing on the disc, especially thick on the marginal portion, with younger ones outwards, rather short in proportion to the size of the actinian. As regards the coloration of the Japanese specimens, they are grouped in the third series by STEPHENSON (1935). They are all brown, grey fawn brown or dark brown. The column is mostly chestnut brown or dark brown

Fig. 30. A part of a colony of *Metridium sensile* var. *fimbriatum* in preserved condition. ×3/2.

and often blotched with lighter colours especially in young specimens. Tentacles greyish brown or brownish blue, with a few yellow distal bands and a white tip, the base being light brown. Acontia white. As was pointed out by MC MURRICH (1901) the brown actinian seems to be the fundamental type, as in the case of *Diadumene Luciae* the olive form with 12 orange stripes is the fundamental one. On account of the occurrence of the asexual reproduction of this species, the numbers of the siphonoglyphes and mesenteries are reported to be variable.

Distribution. This variety has been reported to exist on the Pacific coasts of North America (Puget Sound and Nanaimo) through Alaska, Kamchatka and Behring Sea to Hokkaido (Nemuro). It is common on the coasts of the Kurile Islands, Hokkaido and the northern parts of Honshu in Japan.

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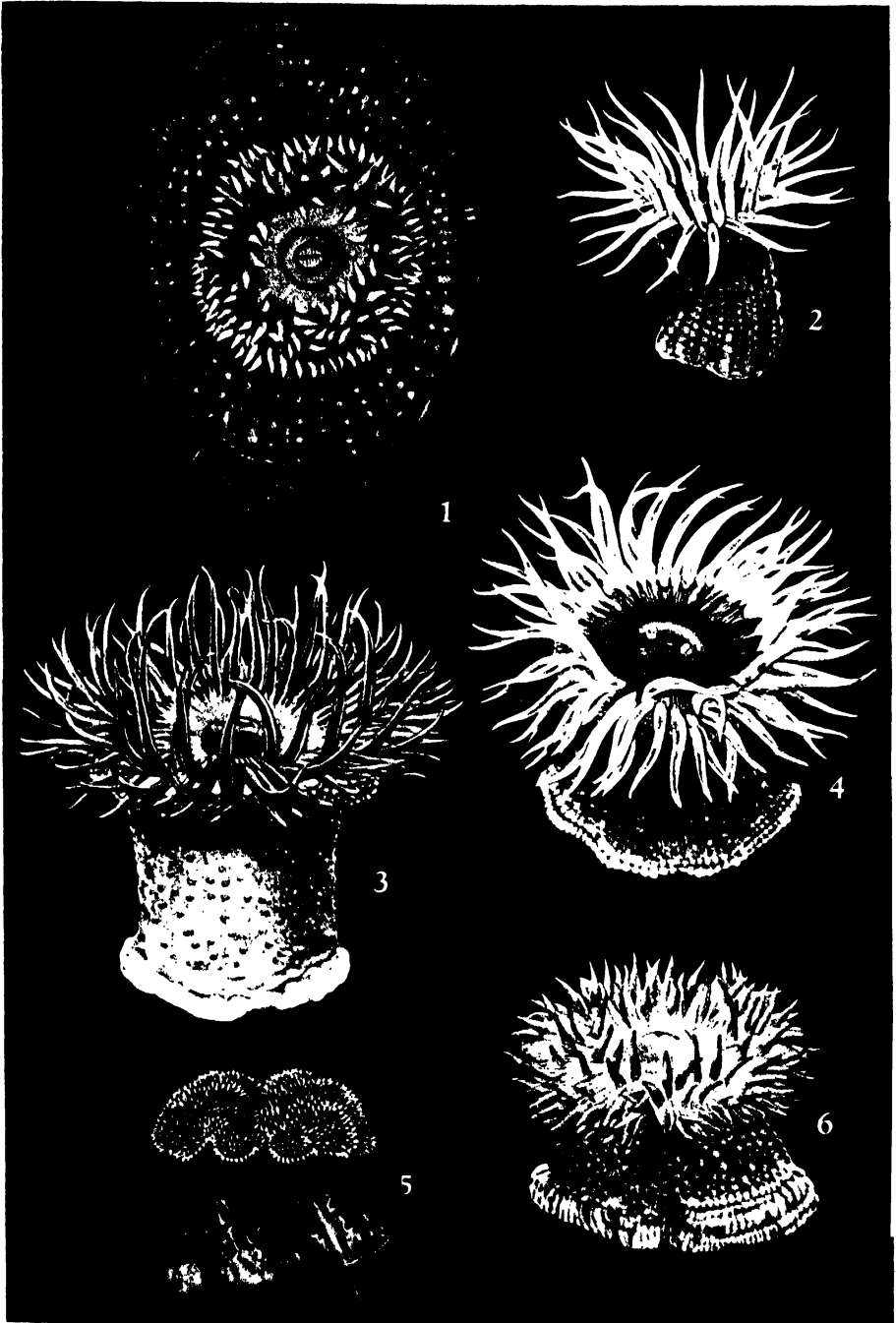
EXPLANATION OF PLATE

1. *Epiactis prolifera* VERRILL
2. *Anthopleura pacifica* n. sp.
3. *Anthopleura japonica* VERRILL
4. *Anthopleura stella* (VERRILL)
5. *Metridium sensile* var. *fimbriatum* VERRILL.
6. *Epiactis prolifera* VERRILL

All in natural size)

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ACTIVE ABSORPTION (IRRECIPROCAL PERMEABILITY) OF Cl' AND ASYMMETRY POTENTIAL ACROSS ISOLATED SKIN OF FROG AND THEIR MODIFICATIONS IN THE ADAPTATION OF THE FROG TO ISOTONIC DILUTED SEA-WATER AND TO DISTILLED WATER[1]

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(With three text-figures)

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CONTENTS

I. INTRODUCTION

The osmo-regulatory mechanism of aquatic organisms has been studied by a large number of investigators (see SCHLIEPER 1930, 1935, ADOLPH 1933, KROGH 1937 *a, b*, 1938, etc.). It is generally accepted that the gills, the skin, the kidney, hypophysis and food are the main factors in this function. In the case of the frog the 4 latter are considered to be concerned with osmo-regulation. In these investigations the frog skin

as the organ of absorption has been the object of the so-called "*irreci-procal permeability*", which means the differing resistance to diffusion in both directions of the membrane (from without inwards and from within outwards). It may be divided into physiological- (due to the active "*Triebkraft*" of the living substance with expenditure of energy), physical- and pseudo-*irreciprocal permeability* (due to the alteration of structure of the transferring substance or to the alteration of structure and physio-logical state of the membrane) (see ECKSTEIN 1936, LEUTHARDT and ZELLER 1934, HUF 1935, RUBINSTEIN and PEVSNER 1937).

According to REID (1890), when normal saline and 5% of glucose in normal saline are used, the normal direction of easier osmotic transference of fluid through the fresh and living skin of the frog is in the direction from without inwards, and not from within outwards, as MATTEUCCI and CIMA (1845) seemed to show; filtration, bagging of the membrane under pressure, and the use of fluids which tended to depress tissue life seem to have been the causes which led these observers into error. Reagents tending to decrease tissue life, such as water and chloroform etc., diminish the amount of fluid passed through the fresh and still living skin, in the direction from without inwards; while, on the other hand, such reagents increase the quantity of fluid passed by osmotic action from within out-wards.

WERTHEIMER (1923 *a*, *b*), using saline and distilled water observed in 2–6 hours experiments the sooner passage of the water in the direction from within outwards, viz. the reverse of the result of REID, while in a 18 hours experiment, the same result as REID's.

PRZYLECKI (1924) using two NaCl solutions (J: 0.37 and 1.07) or the inner NaCl solution (J: 0.47) and the outer (J: 0 and 0.94), respectively, seems to have obtained a result similar to either REID's or WERTHEIMER's.

HEVESY, HOFER and KROGH (1935), using the same Ringer solution except with the different content of D_2O, on both sides of the skin, have shown that the permeability of isolated frog skin and frog legs to heavy water in Ringer is the same in both directions.

RUBINSTEIN and MISKINOWA (1936), using 0.7 Ringer and Ringer, have shown that the irreciprocal permeability to water does not exist and, if it does exist, it is due to experimental error, viz. sooner filtration of liquid from within outwards than in the reverse direction (JURIŠIC 1934) and the application of distilled water, or other solutions which are non-physiological to the inner surface of the skin though HUF (1936 *a*) who used 0.1 Ringer and Ringer was of the opinion that we should not speak

of experimental error but of experimental conditions, and has shown that the easier transport of water is as shown by WERTHEIMER, from the inner towards the outer surface. REY (1938) has noticed that the inward absorption of water begins to decrease in a 1 hour experiment in which the internal surface was exposed to Ringer and the outer, to distilled water, in the case of the skin the internal surface of which was previously in contact with distilled water, 0.25 Ringer, 0.75 Ringer or 1.25 Ringer, respectively, for 1 to 2 hours, as compared with the untreated skin. Using Ringer and distilled water he also observed the more rapid osmotic transport of water in the inward direction in a 3 hours experiment. According to him, these facts seemed to show not the existence of irreciprocal permeability but the modification of the skin caused by the depression of life due to injurious solution; water passes in both directions similarly, in accordance with the osmotic force.

A second experiment is reported by REID (1892) — WERTHEIMER (1923 b) —, in which the same normal saline was placed on both sides of the osmometer, and it was then found that liquid was actively absorbed in the direction from without inwards.

As a result of these experiments (REID 1890, 1892) REID concluded that a satisfactory explanation of the observed facts could only be given on the assumption that there is present in the living skin of the frog a vital absorptive force dependent upon protoplasmic activity and comparable to the vital secretory force of glands. OVERTON (1904) showed that there is an absorption of liquid from a 0.65% NaCl solution in intact frogs. MAXWELL (1913), PRZYLECKI (1924) repeated REID's experiment. According to PRZYLECKI, transferred liquid has the same concentration as that in both solutions. MAXWELL was of opinion that the assumption of the existence of a vital activity in the frog skin in transferring water through itself is unnecessary.

RUBINSTEIN and MISKINOWA (1936), RUBINSTEIN and PEVSNER (1937) assigned REID's experiment (1892) to experimental error due to the application of skin when held between two baudruche membranes instead of applying the bare skin itself. HUF (1935 a, b, 1936 a, b, c) re-proved REID's experiment against its negation (JURIŠIČ 1931 II, III, 1936 a) and furthermore observed against PRZYLECKI, the concentration work with reference to Cl′.

As regards Cl′ PRZYLECKI (1924), WERTHEIMER (1923 a), HUF (1936), and others have reported the inward permeability and transference; ADOLPH (1927, 1933), GRAETZ (1934), KROGH (1937, 1938) and REY

(1938), using an intact frog, have proved the active absorption of Cl′. KROGH (1937 a) has even found that the loss of Cl′ through the skin in a frog, constantly exposed to a spray of distilled water, becomes very slight, while the concentration of the urine also falls, and that frogs sprayed for some time with distilled water will absorb Cl′ through the skin even from a very diluted solution of NaCl, corresponding to $\frac{\text{Ringer}}{100}$; the biological significance of the Cl′-absorption, which is well developed in the majority of fresh-water vertebrates and invertebrates has been discussed by KROGH (1937 a, b, 1938). The occurrence of absorption or excretion (or irreciprocal permeability) has been sometimes discussed in connection with asymmetric properties, especially the asymmetric potential for example by JURIŠIĆ (1931 a), KELLER (1931), MOND (1927), AMSON (1930), LEUTHARDT and ZELLER (1934), HUF (1935 a), RUBINSTEIN and PEVSNER (1937), WILBRANDT (1938), etc., and with respiration (WOWSI 1932, HUF 1935 a). The potential has also been considered to depend on respiration (LUND 1928, FRANCIS 1934, TAYLOR 1935, PONDER and MACLEOD 1936 etc.); The asymmetry potential of frog skin has been studied by many investigators (for example HASHIDA 1922, UHLENBRUCK 1924, STEINBACH 1933, MOTOKAWA 1935) since DU BOIS-REYMOND (1857).

The adaptation of the frog to diluted sea-water or to Ringer's solution has also been investigated by some authorities (BERTIN 1920, DUVAL 1928, REY 1938).

II. MATERIAL AND TECHNIQUE

1. Frog and its skin. The frogs used were *Rana nigromaculata nigromaculata* (HALLOWELL). They were put under running tap water, kept without food after capture, and acclimatized at an experimental temperature for from 1 to 12 hours before being struck on the head and decapitated, and the ventral skin was then removed, which was sometimes washed with isotonic sea-water and wiped with filter paper before use. The isolated skin will survive for 2 days or more after its removal from the body in Ringer or isotonic sea-water (Table I). This has been ascertained by many authorities by measuring its oxygen consumption (ABDERHALDEN and WERTHEIMER 1921), or its electerical resistance (SCHWARTZ 1913), or the electrical potential difference, which its sides maintain (DU BOIS-REYMOND 1857), or by noting change of permeability (PRZYLECKI 1924). It has however been ascertained that the disappearance of one of these results does not necessarily accompany any of the others (for

example PONDER and MACLEOD 1936; RUBINSTEIN and PEVSNER 1937, etc.).

In the present investigation, the potential was sometimes measured parallel with permeability (absorption).

2. *Apparatuses for permeability-studies.* As shown in Fig. 1, to the lower end of a graduated tube 2.1 and 0.6 cm in diameter at the lower end and upper uniform part, respectively, were tightly fastened with cotton thread two sheets of 2 mm mesh cotton-net, between which the frog skin was spread. The sides of this tube, on and between each layer, were coated with a thick or thin film of vaseline, respectively. 2 cc. of a solution (inner solution) were measured accurately into the tube with an ordinary certified full-pipette, and the tube was shaken to remove air bubbles. Then the meniscus was read. After the stirrer had been dipped into and taken out of the solution, being rotated and rubbed against the inner wall of the tube so that the amount of solution adhering to it might be as small as possible, the volume was again read. The tubes was dipped into a solution (about 3 L.) in a glass jar through holes in its lid, to which they were fixed by a gum stopper so as to equalized the hydrostatic pressure. In order to renew the solution in the jar (outer solution) a new solution of the same concentration was dropped into it, and the excess flowed out of it at the rate of 0.5 L. per hour. The maintenance of the constant pH, oxygen supply and stirring were kept steady

Fig. 1.

by CO_2-free air bubbling by means of an alkali-containing wash bottle and an air-blower, which consisted of an aspirator and a bottle. In these conditions, the concentration of the outer solution was unchanged in spite of the exchange of water and ions between the inner and outer solutions. The inner solution was stirred gently by a glass stirrer. The whole apparatus was placed in a thermostat. Before the experiment, the preparation was kept in the same experimental condition for 10 to 30 minutes.

3. *Respirometry.* Oxygen was determined by the micro-WINKLER method (RISCH 1925). 10 cc. of the sample solution was used; 0.005 N
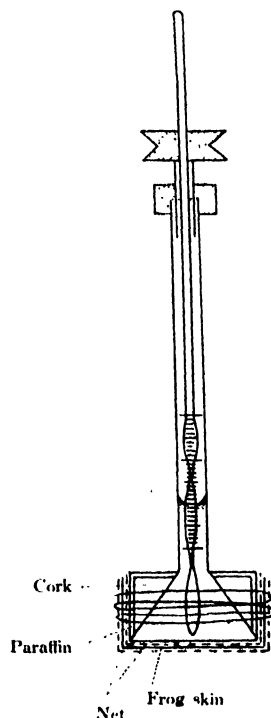
thiosulphate was also used.

4. *Determination of Cl'.* The micro-VOLHARD method was used; 0.05 N AgNO₃ was standardized against 0.05 NaCl; ether was employed to coagulate AgCl-precipitates.

5. *Preparation of the experimental solutions.* Diluted sea-water isotonic with the frog is a desirable medium for the frog's heart (see ZIGANOW 1926). As the survival of the frog skin was longer in diluted sea-water isotonic with the frog than in Ringer (Table I), I used isotonic sea-water instead of Ringer; frog Ringer: NaCl 6.5 g., KCl 0.2 g., CaCl₂ 0.2 g. per L., $\varDelta$: -0.405; isotonic diluted sea-water (sea-water Ringer: abbreviated into R_s): Cl: 4.2-4.5 g. per L., $\varDelta$: -0.43-0.46.

TABLE I.

Potential Differences of the Skin of the Frog in isotonic Sea-water and in Ringer's Solution.

External surface of the skin $(-)$; 20.0°C

Time hrs.	Inner & outer solution =dil. sea-water ($\Delta = -0.43$)		Inner & outer solution =Ringer's solution ($\Delta = -0.405$)
	April 8, '37		July 10, '37
	Frog a ♂ Volt.	Frog b ♂ Volt.	Frog c ♂ Volt.
0	0.0781	0.0410	0.0380
½	-	--	0.0320
1	-	--	0.0390
2	--	-	0.0410
3	0.0540	---	0.0485*
15	0.0440*	0.0430*	0.0480
20	0.0411	0.0460	
23	0.0390	---	
25		-	0.034
26	---	- -	0.039*
39	0.0291*	0.0370*	-
44	....	-	0.010
47	0.0211	---	---
64	0.0111*	0.0171*	-
66	0.0105	- -	- -
70	0.0060	0.0111	-

* Both solutions were renewed.

The relation between the chloride ion concentration of sea-water and the freezing point depression was determined and compared with that calculated from the table given in Tabulae biologicae (IV, 1927) and compiled in Table II.

<div align="center">

TABLE II.

Relation between Freezing Point Depression and Cl'
Concentration of Sea-water.

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Salt content ‰	Cl' g. p. 100 cc.	Δ °C	|Cl'| − |Δ|	Remarks
	1.886	−1.87	+0.02	
	1.257	−1.24	+0.02	
	0.943	−0.935	+0.00	Sea-water
	0.499	−0.50	−0.00	(Asamushi)
	0.480	−0.49	−0.01	Sept. 2, 1936.
	0.412	−0.42	−0.01	
34	1.900	−1.853	+0.047	
33	1.841	−1.797	+0.044	Recalculated
22	1.237	−1.184	+0.053	from " Tabulae
17	0.952	−0.910	+0.042	Biologicae "
9	0.500	−0.480	+0.020	
8	0.444	−0.427	+0.017	Cl' (15.0°C)

6. *Potential measurements.* R_s was placed, in the tube with the skin previously described, or in a small glass tube with the skin; this tube was plunged into the R_s contained in a vessel, so as to equalize the hydrostatic pressure; another R_s and R_s-bridges were introduced between the saturated KCl solution, into which was dipped a calomel half-cell saturated with KCl, and R_s, which was in direct contact with the skin membrane, in order that the membrane might not be contaminated with KCl from the KCl-agar-bridges. Thus the chain the EMF of which was measured, was the following:

$$Hg \begin{vmatrix} HgCl \\ KCl \\ sat. \end{vmatrix} \begin{vmatrix} KCl \\ sat. \end{vmatrix} \begin{vmatrix} Agar \\ brid. \end{vmatrix} R_s \begin{vmatrix} R_s \\ brid. \end{vmatrix} R_s |Skin| R_s \begin{vmatrix} R_s \\ brid. \end{vmatrix} R_s \begin{vmatrix} Agar \\ brid. \end{vmatrix} \begin{vmatrix} KCl \\ sat. \end{vmatrix} \begin{vmatrix} HgCl \\ KCl \\ sat. \end{vmatrix} Hg$$
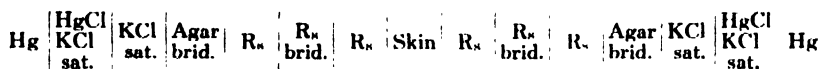
Two calomel half-cells were checked against each other, before the experiment, and correction for potential difference was made. The potentials were measured in the usual way, with a high-resistance galvanometer as a null instrument.

<div align="center">

III. RESULTS, DISCUSSION AND CONCLUSIONS

</div>

1. *Active absorption of Cl' through the isolated skin, when its*
internal surface is exposed to isotonic sea-water and its outer
(epithelial side) to various concentrations of sea-water.

Using the isotonic sea-water (R_s) as the solution in contact with the inner surface of the skin and various concentrations from $\frac{R_s}{100}$ to $2 R_s$

as the solution exposed to the outer surface, the results embodied in Tables IV, V, VI, VII, VIII and X (see also Table IX) prove that there is an active absorption of Cl' from without (epithelial side) inwards through fresh frog skin, even in opposition to osmotic pressure. This phenomenon took place in great quantities in a high concentration and in small quantities in diluted solutions. A similar case in intact frogs was dealt with by ADOLPH (1927, 1933). In the case of the diluted medium $\left(\frac{R_н}{10}\right)$, absorption seemed to take place during the early stage of the experiment. Into tap water or $\frac{R_н}{100}$, outward Cl'-diffusion was practically O or very slight (Table IX and XXI) (see HUKUDA & WATANABE 1930). The vitality of the skin was ascertained by measuring the skin potential:

Maintenance

$\begin{cases} R_н \text{ to inner surface} \\ \text{Tap water to outer surface} \end{cases}$: (normal condition) 24 hrs. at least (Table IX)

$\begin{cases} R_н \text{ to inner surface} \\ R_. \text{ to outer surface} \end{cases}$ .... 2 days (Table I)

$\begin{cases} R_н \text{ to inner surface} \\ 2 R_н \text{ to outer surface} \end{cases}$ .... 5–(10) hrs. (Table III)

$\begin{cases} 1.5 R_н \text{ to inner surface} \\ R_{..} \text{ to outer surface} \end{cases}$ .... 23 hrs. (Table III)

$\begin{cases} \frac{1}{2} R_н \text{ to inner surface} \\ R_. \text{ to outer surface} \end{cases}$ .... 12 hrs. (Table III)

TABLE III.

Skin Potential after Treatment with various Solutions.

1. *Skin with inner Surface bathed in $R_.$ and outer Surface in $2 R_н$.*

    July 16, 1938. 20.0°C. The outer surface was exposed to outer solution.

         ♀   0.037 v —————→ (0.004 ——— ——→ 0.005 v ——— —→ 0.000 v
              Both R_3 I.S $R_н$ Both R_3 I.S. $R_н$ Both $R_.$
                        O.S. 2 $R_н$ O.S. 2 $R_.$

         ♂   0.430 v ——————→ (0.0180 ———————→ 0.0250 v) ——— —→ 0.0000 v
              Both=R_4 I.S. $R_.$ Both $R_н$ I.S. $R_.$ Both $R_.$
                         O.S. 2 $R_.$ O.S. 2 $R_.$

    August 2, 1938. 21.0 C; caught: July 31

         ♀   0.0455 v ————→ (0.0050 ————————→ 0.0030 v)
              Both R_3 I.S. $R_н$ Both $R_.$
                        O.S.=2 $R_.$

         ♀   0.03620 v ———— →(0.0032 ——— ——→ 0.0040 v)
              Both $R_.$ I.S. $R_н$ Both $R_.$
                        O.S. 2 R_3

2. *Skin with inner Surface bathed in* $\dfrac{R_s}{2}$ *and outer Surface in* R_s.

Sept. 20, 1937. 20.0°C.

The inner surface was exposed to the outer solution.

        * 12 hrs. 20 mins. 7^h10^m 1^h10^m 5 hrs. 44 mins.

♀ 0.046 v ⟶(0.021 ⟶ 0.055 v)⟶(0.0009 ⟶ 0.051 v)⟶(0.005 ⟶ 0.0175 v)

 Both R_s I. S.=R_s Both=R_s I. S.=R_s Both R_s I. S. R_s Both R_s

     O.S.=$\dfrac{R_s}{2}$ O.S.=$\dfrac{R_s}{2}$ O.S.=$\dfrac{R_s}{2}$

        * 12 hrs. 30 mins.   9 hrs. 1^h30^m

♀ 0.052 v ⟶(0.005 ⟶ 0.0190 v)⟶(0.003 ⟶ 0.0127 v)

 Both=R_s I. S.=R_s Both=R_s   I. S. R_s   Both R_s

     O.S.=$\dfrac{R_s}{2}$ O.S.=$\dfrac{R_s}{2}$

3. *Skin with inner Surface bathed in* 1.5 R_s *and outer Surface in* R_s.

July 14, 1938. 20.0°C.

The outer surface was exposed to the outer solution.

        * 23 hrs. 50 mins.   * 24 hrs.

♀ 0.042 v ⟶(0.0255 ⟶ 0.018 v); ♀ 0.0392 v ⟶ 0.005 v

 Both R_s I. S. 1.5 R_s Both R_s   Both R_s I. S. 1.5 R_s Both R_s

     O.S. R_s   O.S. R_s

4. *Skin with inner Surface bathed in* $\dfrac{1}{5}$ R_s *and outer Surface in* R_s.

Nov. 26, 1937. 20.0°C.

The inner surface was exposed to the outer solution.

        * 1 hr.   1 hr.   1 hr.   1 hr.   1 hr.

♀ 0.0524 v ⟶(0.00315 ⟶ 0.0130 ⟶ 0.0281 ⟶ 0.0396 ⟶ 0.0413 v)

 Both R_s I. S.=R_s

     O.S.=$\dfrac{R_s}{5}$ Both=R_s

---

* Not initial potential but potential which became constant after some time.

TABLE IV.

Transference of Cl′ through isolated Skin.

Inner solution = R_s 2 cc.

Outer solution = 2 R_s ∞ cc.

Temp. = 20.0°C.

The external surface was exposed to the outer solution.

R_s

2 R_s H_2O Cl′

- - - : Internal surface

——— : External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner sol. g.-eq. p. l.	Water increased in inner sol.		Cl′ increased in inner sol.		Remarks
					cc.	%	m.-eq.	%	
10	Oct. 10, 1937	1	5 { 5.51 p.m. / 10.51 "	0.1550	−0.235	−11.8	+0.0176	+ 6.9	I. S. = 0.1280 N. Cl′ O.S. = 0.2560 N. Cl′
		2	5 { 11.16 p.m. / 4.16 a.m.	0.1560	−0.100	− 5.0	+0.0404	+15.8	
		3	5 { 4.36 a.m. / 9.36 "	0.1583	−0.080	− 4.0	+0.0479	+18.7	
		4	5 { 9.55 a.m. / 2.55 p.m.	0.1610	−0.075	− 3.8	+0.0539	+21.1	
		5	5 { 3.22 p.m. / 8.22 "	0.1598	−0.070	− 3.5	+0.0524	+20.5	

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol.		Cl' increased in inner sol.		Remarks
					cc.	%	m.-eq.	%	
11	Oct. 10, 1937	1	5{ 6.20 p.m. / 11.20 ,,	0.1535	−0.125	−6.25	+0.0318	+12.4	I. S. = 0.1280 N. Cl' O.S. = 0.2560 N. Cl'
		2	5{ 11.48 p.m. / 4.48 a.m.	0.1591	−0.100	−5.0	+0.0462	+18.1	
		3	5{ 5.04 a.m. / 10.04 ,,	0.1601	−0.080	−4.0	+0.0514	+20.1	
		4	5{ 10.22 a.m. / 3.22 ,,	0.1654	−0.080	−4.0	+0.0615	+24.0	
		5	5{ 3.34 p.m. / 8.34 ,,	0.1664	−0.090	−4.5	+0.0618	+24.1	

TABLE V.

Transference of Cl' through isolated Skin.

Inner solution = R_s 2 cc.
Outer solution = R_s ∞ cc.
Temp. = 20.0°C.

R_s $\downarrow$ $\downarrow$ R_N $\downarrow$

R_s H_2O Cl' R_N H_2O Cl'

- - - : Internal surface
——— : External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol.		Cl' increased in inner sol.		Surface exposed to outer solution	Remarks
					cc.	%	m.-eq.	%		
↑ 25	Nov. 30, 1937	1	5{ 0.35 p.m. / 5.35	0.1285	+0.002	+0.1	+0.0043	+1.7	External	$R_s = 0.1265$ N. Cl'
		2	5{ 6.03 p.m. / 11.03 ,,	0.1287	+0.013	+0.65	+0.0061	+2.4		
		3	5{ 11.34 p.m. / 4.34 a.m.	0.1277	+0.010	+0.5	+0.0037	+1.5		
		4	5{ 5.17 a.m. / 10.17 ,,	0.1287	+0.010	+0.5	+0.0057	+2.3		
↑ 26		1	5{ 0.49 p.m. / 5.49 ,,	0.1252	−0.010	−0.5	−0.0038	−1.5	Internal	
		2	5{ 6.21 p.m. / 11.21 ,,	0.1254	−0.005	−0.25	−0.0026	−1.0		
		3	5{ 11.56 p.m. / 4.56 a.m.	0.1259	−0.010	−0.5	−0.0025	−1.0		
		4	5{ 5.45 a.m. / 10.45 ,,	0.1257	±0	±0	−0.0015	−0.6		
♀ 27	Dec. 3, 1937	1	10{ 10.51 a.m. / 8.51 p.m.	0.1247	−0.015	−0.75	−0.0044	−1.8	Internal	$R_s = 0.1260$ N. Cl'
		2	10{ 9.25 p.m. / 7.25 a.m.	0.1255	−0.005	−0.25	−0.0016	−0.6		
		3	10{ 7.25 a.m. / 5.25 p.m.	0.1260	−0	0	0	0		

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner sol. g.-eq. p. L.	Water increased in inner sol.		Cl′ increased in inner sol.		Surface exposed to outer solution	Remarks
					cc.	%	m.-eq.	%		
♂↑28	Dec. 3. 1937	1	10{11.12 a.m. / 9.12 p.m.}	0.1278	+0.005	+0.25	+0.0042	+1.7		R.=0.1260 N. Cl′
		2	10{9.54 p.m. / 7.54 a.m.}	0.1267	+0.008	+0.4	+0.0025	+1.0	External	
		3	10{8.16 a.m. / 6.16 p.m.}	0.1265	+0.003	+0.15	+0.0030	+1.2		

TABLE VI.

Transference of Cl′ through isolated Skin.

Outer solution $= \frac{1}{2}$ R. ∞ cc.

Inner solution = R. 2 cc.

Temp. = 20.0°C

The external surface was exposed to the outer solution.
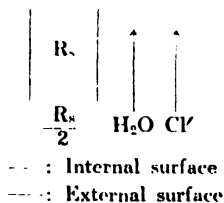
--- : Internal surface

---- : External surface

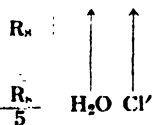| Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner sol. g.-eq. p. L.	Water increased in inner sol.		Cl′ increased in inner sol.		Remarks
					cc.	%	m.-eq.	%	
♀15	Oct. 21, 1937	1	5{0.42 p.m. / 5.42 ,,}	0.1210	+0.145	+7.25	+0.0024	+0.9	I.S.=0.1285 N. Cl′ O.S.=0.0643 N. Cl′
		2	5{6.06 p.m. / 11.06 ,,}	0.1220	+0.105	+5.52	+0.0046	+1.8	
		3	5{11.42 p.m. / 4.42 a.m.}	0.1240	+0.075	+3.75	+0.0003	+0.1	
		4	5{5.07 a.m. / 10.07 ,,}	0.1250	+0.070	+3.5	+0.0017	+0.7	
♂16		1	5{0.48 p.m. / 5.48 ,,}	0.1219	+0.115	+5.75	+0.0007	+0.3	
		2	5{6.16 p.m. / 11.16 ,,}	0.1219	+0.140	+7.0	+0.0038	+1.5	
		3	5{11.59 p.m. / 4.59 a.m.}	0.1212	+0.140	+7.0	+0.0024	+0.9	
		4	5{5.22 a.m. / 10.22 ,,}	0.1222	+0.115	+5.75	+0.0015	+0.6	

TABLE VII.

Transference of Cl' through isolated Skin.

Outer solution $= \frac{1}{5}$ R$_N$ ∞ cc.

Inner solution $=$ R$_i$ 2 cc.

Temp. $= 20.0°$C

The external surface was exposed to the outer solution.

$\frac{R_N}{5}$ H$_2$O Cl'

----: Internal surface
——: External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	%	Cl' increased in inner sol. m.-eq.	%	Remarks
♀ 17	Oct. 26, 1937	1	5{ 0.56 p.m. / 5.56 ,,	0.1229	+0.110	+5.5	+0.0024	+0.9	I. S. = 0.1285 N. Cl' O.S. = 0.02552 N. Cl'
		2	5{ 6.20 p.m. / 11.20 ,,	0.1225	+0.100	+5.0	+0.000(2)	+0.1	
		3	5{ 0.00 a.m. / 5.00 ,,	0.1215	+0.115	+5.75	+0.000(1)	+0.0	
		4	5{ 5.14 a.m. / 10.14 ,,	0.1217	+0.105	+5.25	+0.000	±0.0	
♂ 18		1	5{ 0.45 p.m. / 5.45 ,,	0.1237	+0.108	+5.4	+0.0038	+1.5	
		2	5{ 6.09 p.m. / 11.09 ,,	0.1246	+0.080	+4.0	+0.0021	+0.8	
		3	5{ 11.39 p.m. / 4.39 ,,	0.1250	+0.070	+3.5	+0.0017	+0.7	
		4	5{ 4.58 a.m. / 9.58 ,,	0.1242	+0.065	+3.25	−0.0005	−0.2	

TABLE VIII.

Transference of Cl' through isolated Skin.

Inner solution $=$ R$_i$ 2 cc.

Outer solution $= \frac{R_N}{10}$ ∞ cc.

Temp. $= 20.0°$C

The external surface was exposed to the outer solution.

$\frac{R_N}{10}$ H$_2$O Cl'

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	%	Cl' increased in inner sol. m.-eq.	%	Remarks
♀ 19	Oct. 29, 1937	1	5{ 0.28 p.m. / 5.28 ,,	0.1151	+0.250	+12.5	+0.0020	+0.8	I. S. = 0.1285 N. Cl' O.S. = 0.01285 N. Cl'
		2	5{ 6.01 p.m. / 11.01 ,,	0.1179	+0.175	+8.75	−0.0006	−0.2	
		3	5{ 11.43 p.m. / 4.43 a.m.	0.1181	+0.180	+9.0	+0.0005	+0.2	
		4	5{ 5.20 a.m. / 10.20 ,,	0.1188	+0.155	+7.75	−0.0010	−0.4	

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	%	Cl' increased in inner sol. m.-eq.	%	Remarks
♀ 20	Oct. 29, 1937	1	5{ 1.09 p.m. / 6.09 ,,	0.1177	+0.230	+11.5	+0.0055	+2.1	I.S.=0.1285 N. Cl' O.S.=0.01285 N. Cl'
		2	5{ 6.22 p.m. / 11.22 ,,	0.1192	+0.155	+7.75	0.0001	−0.0	
		3	5{ 0.00 a.m. / 5.00 ,,	0.1197	+0.135	+6.75	−0.0014	−0.5	

TABLE IX.

Transference of Cl' through isolated Skin.

Inner solution = R_N 2 cc.
Outer solution = Tap water
(Cl'≠0.001 N) ∞ cc.

R_N R_N

T   H₂O Cl'   T   H₂O Cl'

- - - - : Internal surface
———— : External surface

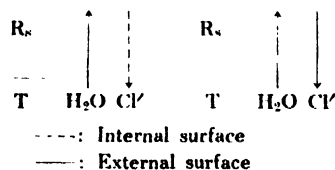| Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	%	Cl' increased in inner sol. m.-eq.	%	Surface exposed to outer sol.	Remarks
♀ 1	June 24-25, 1937	1	5{ 6.11 p.m. / 11.11 ,,	0.1197	+0.14	+7.0	+0.000(2)	+0.1	External	Temp.=20.0°C I.S.=0.1280 N. Cl'
		2	5{ 11.40 p.m. / 4.40 a.m.	0.1217	+0.09	+4.5	+0.001(6)	−0.6		
		3	5{ 5.06 a.m. / 10.06 ,,	0.1220	+0.07	+3.5	+0.003(5)	−1.3		
♀ 2		1	5{ 6.50 p.m. / 11.50 ,,	0.0971	+0.16	+8.0	−0.046(3)	−18.1	Internal	
		2	5{ 0.14 a.m. / 5.14 ,,	0.0825	+0.15	+7.5	−0.078(6)	−30.7		
		3	5{ 5.35 a.m. / 10.35 ,,	0.0971	+0.17	+8.5	−0.045(3)	−17.7		
♀ 57	May 27, 1938	1	5{ 0.07 p.m. / 4.37 ,,	0.1236	+0.050	+2.5	−0.000(4)	−0.2	External	Temp.=20.0°C I.S.=0.1269 N. Cl' Collection: March 6
		2	5{ 4.49 p.m. / 9.19 ,,	0.1241	+0.047	+2.4	+0.000(3)	−0.1		
		3	11½{ 9.32 p.m. / 9.02 a.m.	0.1186	+0.095	+4.75	−0.0050	−2.1		
♀ 59	May 31, 1938	1	5{ 9.15 a.m. / 2.15 p.m.	0.1226	+0.067	+3.4	−0.0008	−0.3	External	Temp.=20.0°C I.S.=0.1272N. Cl' Final skin potential=0.0065 v Collection: March 6
		2	5{ 2.30 p.m. / 7.30 ,,	0.1229	+0.055	+2.8	−0.0016	−0.6		
		3	11{ 8.04 p.m. / 7.04 a.m.	0.1181	+0.095	+4.8	−0.0068	−2.7		

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner sol. g.-eq. p. L.	Water increased in inner sol.		Cl′ increased in inner sol.		Surface exposed to outer sol.	Remarks
					cc.	%	m.-eq.	%		
→66	July 27, 1933	1	5{ 9.29 a.m. / 2.29 p.m.	0.1225	+0.055	+2.8	−0.0024	−0.9	External	Temp.=21.0°C I.S.=0.1271N.Cl′ Final skin potential=0.0142 v Collection: June 27
		2	5{ 2.44 p.m. / 7.44 ,,	0.1234	+0.050	+2.5	−0.0012	−0.5		
		3	10{ 8.25 p.m. / 6.25 a.m.	0.1172	+0.110	+5.5	−0.0069	−2.7		

TABLE X.

Transference of Cl′ through isolated Skin.

Inner solution = R_N (Cl = 0.1270 N) 2 cc.

Outer solution = 1.5 R_N (Cl = 0.1913 N) ∞ cc.

The internal surface was exposed to the outer solution.

R_N

1.5 R_N H_2O Cl′
- - - - : Internal surface
— · : External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner solution g.-eq. p. L.	Water increased in inner sol.		Cl′ increased in inner sol.	
					cc.	%	m.-eq.	%
→21		1	5{ 0.30 p.m. / 5.30 ,,	0.1353	−0.130	−6.5	−0.0009	−0.4
		.2	5{ 5.54 p.m. / 10.53 ,,	0.1362	−0.150	−7.5	−0.0019	−0.8
		3	5{ 11.18 p.m. / 4.18 a.m	0.1374	· 0.140	−7.0	+0.0016	+0.6
		4	5{ 4.46 a.m. / 9.46 ,,	0.1389	−0.120	6.0	+0.0071	+2.8
	Nov. 11, 1937	5	5{ 10.16 a.m. / 3.16 p.m.	0.1394	−0.095	−4.8	+0.0115	+4.5
♀22		1	5{ 1.14 p.m. / 6.14 ,,	0.1318	−0.090	−4.5	−0.0023	−0.9
		2	5{ 6.32 p.m. / 11.32 ,,	0.1316	−0.070	−3.5	±0	±0
		3	5{ 11.54 p.m. / 4.54 a.m.	0.1316	−0.073	−3.7	· 0.0005	0.2
		4	5{ 5.16 a.m. / 10.16 ,,	0.1321	−0.075	−3.8	+0.0002	+0.1
		5	5{ 10.35 a.m. / 3.35 p.m.	0.1318	−0.073	−3.7	+0.0004	+0.2

According to REY (1938), HEVESY, HOFER and KROGH (1935), RUBINSTEIN and MISKINOWA (1936), as seen in introduction, no force but osmotic pressure seemed to control the passage of the water through the isolated skin, viz. there is no active absorption of water or irreciprocal permeability.

Though Table XI seems to indicate the sooner osmosis of water from without inwards, it is not necessary to assume the existence of an active

TABLE XI.

Transference of Water through isolated Skin.

Inner solution $= R_s$ 2 cc.;   Outer solution $- \dfrac{R_s}{2} \sim$ cc.   Temp. $= 20.0°C$.

Frog No.	Date	Exp. No.	Time hrs.	Water inc. in inner sol. cc.	Surface exposed to outer solution	Remarks
♀ 13	Oct. 14, 1937	1	3 { 10.40 a.m. / 1.40 p.m.	+0.125	External	Frogs, 23 days in tap water since collection
		2	3 { 2.15 „ / 5.15 „	+0.065	Internal	
		3	3 { 5.51 „ / 8.51 „	+0.077	External	
♀ 14		1	3 { 11.45 a.m. / 2.45 p.m.	+0.030	Internal	
		2	3 { 3.16 „ / 6.16 „	+0.075	External	
		3	3 { 6.25 „ / 9.25 „	+0.050	Internal	
♀ 94	Oct. 22, 1938	1	2 { 10.17 a.m. / 0.17 p.m.	+0.045	External	Frogs, 2 days in tap water since collection
		2	2 { 0.39 „ / 2.39 „	+0.020	Internal	
		3	2 { 3.06 „ / 5.06 „	+0.039	External	
♂ 95		1	2 { 11.05 a.m. / 1.05 p.m.	+0.035	Internal	
		2	2 { 1.36 „ / 3.36 „	+0.030	External	
		3	2 { 4.04 „ / 6.04 „	+0.018	Internal	
♀ 98	Oct. 26, 1938	1	2 { 10.25 a.m. / 0.25 p.m.	+0.018	Internal	Frogs, 2 days in tap water since collection
		2	2 { 1.16 „ / 3.16 „	+0.020	External	
		3	2 { 4.01 „ / 5.01 „	+0.018	Internal	
♀ 99		1	2 { 11.03 a.m. / 0.03 p.m.	+0.022	Internal	
		2	2 { 1.30 „ / 3.30 „	+0.030	External	
		3	2 { 4.19 „ / 6.19 „	+0.025	Internal	

absorptive force or irreciprocal permeability. That is, as shown in Fig. 2, in (a) internal concentration tends to increase owing to active absorption of Cl′ while in (b) it tends to decrease so that in the former water streams more quickly from the outer towards the inner solution than in the latter.
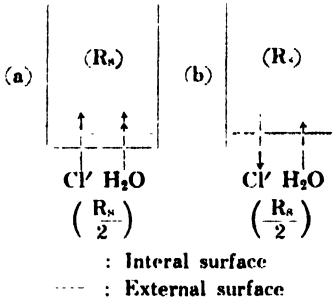

: Interal surface
---- : External surface

Fig. 2.

If the rate at which water passed through, when the outer side was exposed to the tap water or $\frac{1}{5}$ R$_x$ and the internal surface to R$_x$, was compared with that when the orientation of the skin was reversed, the transfer of the liquid took place most readily from within outwards (epithelial side) (Tables IX, XII). This is probably a pseudo-irreciprocal permeability due to alteration of the skin, caused by the non-physiological conditions in which the inner surface of the skin faces the tap water or $\frac{1}{5}$ R$_x$ (Table III 4). The irreciprocal permeability of frog skin to water found by WERTHEIMER (1923 a), was based on this phenomenon and others; BAUER (1925), especially RUBINSTEIN and MISKINOWA (1936) attributed it to experimental error, viz. to deviation from physio-

TABLE XII.

Transference of Water through isolated Skin.

Inner solution = R$_x$ 2 cc.;   Outer solution = $\frac{R_x}{5}$ ∞ cc.;   Temp. = 20.0°C.

Frog No.	Date	Exp. No.	Time hrs.	Water inc. in inner sol. cc.	Surface exposed to outer solution	Remarks
♂ 84		1	2 { 10.36 a.m. / 0.36 p.m.	0.070	External	
		2	2 { 1.07 ,, / 3.07 ,,	0.045	Internal	
	Oct. 17, 1938	3	2 { 3.37 ,, / 5.37 ,,	0.028	External	Frogs, 24 days in tap water since collection
♀ 85		1	2 { 11.17 a.m. / 1.17 p.m.	0.045	Internal	
		2	2 { 1.46 ,, / 3.46 ,,	0.025	External	
		3	2 { 4.14 ,, / 6.14 ,,	0.045	Internal	

Frog No.	Date	Exp. No.	Time hrs.	Water inc. in inner sol. cc.	Surface exposed to outer solution	Remarks
♀ 90		1	1⅙ {11.09 a.m. / 0.19 p.m.}	0.015	External	
		2	1⅙ {0.57 ,, / 1.07 ,,}	0.023	Internal	
		3	1⅙ {2.42 ,, / 3.52 ,,}	0.013	External	
	Oct. 20, 1938	4	1⅙ {4.20 ,, / 5.30 ,,}	0.025	Internal	Frogs, 27 days in tap water since collection
♀ 91		1	1⅙ {10.20 a.m. / 11.30 ,,}	0.035	Internal	
		2	1⅙ {0.02 p.m. / 1.12 ,,}	0.027	External	
		3	1⅙ {1.43 ,, / 2.53 ,,}	0.035	Internal	
		4	1⅙ {3.27 ,, / 4.37 ,,}	0.025	External	
♀ 92		1	1 {10.11 a.m. / 11.11 ,,}	0.015	External	
		2	1 {11.19 ,, / 0.19 p.m.}	0.013	,,	
		3	1 {1.00 ,, / 2.00 ,,}	0.015	Internal	
		4	1 {2.38 ,, / 3.38 ,,}	0.012	External	
	Oct. 21, 1938	5	1 {4.17 ,, / 5.17 ,,}	0.016	Internal	Frogs, one day in tap water since collection
♀ 93		1	1 {10.47 a.m. / 11.47 ,,}	0.015	External	
		2	1 {11.57 ,, / 0.57 p.m.}	0.020	,,	
		3	1 {1.25 ,, / 2.25 ,,}	0.027	Internal	
		4	1 {3.06 ,, / 4.06 ,,}	0.010	External	
		5	1 {4.26 ,, / 5.26 ,,}	0.020	Internal	

logical condition; HUF (1936 a), using Ringer and 0.1 Ringer and changing the orientation of the same skin every hour, re-proved that the osmosis of water in the direction from the internal side towards the outer occurs more rapidly than in the reverse direction; and he is of opinion that we should not speak of experimental error but experimental conditions.

Using a 20% cane-sugar solution in water, water and the Dutrochet osmometer, REID (1890) found that during the earlier stages of the

experiment (ca. 5 hours) the stream from without (epithelial side) inwards is the more rapid of the two, while in the later stages the stream from within outwards exceeds that in the reverse direction. When 5% glucose in normal saline and normal saline were used, the direction of easier passage of the fluid was from without inwards, which was maintained during the whole course of the experiment (24 hours).

These results may be due to the difference of conditions and to the behaviour of the internal and outer surfaces in contact with the applied solutions and to the alteration of concentration which is caused by the active absorption of Cl' in the direction from the outer to internal surface ; that is, 20% sugar is injurious to the internal surface because of the non-existence of electrolyte and the hypertonicity ; water is not injurious to the outer surface but to the inner. 5% glucose in normal saline is hyper-

S_n: Normal saline
g: Glucose

: Internal surface
: External surface

Fig. 3.

tonic and, therefore, not a better solution and, as shown in Fig. 3, in (a) internal concentration tends to increase owing to active absorption of Cl' while in (b) it tends to decrease so that in the former water streams more quickly from the outer towards the inner solution than in the latter.

REID's 2nd experiment (1892) — MAXWELL (1913), WERTHEIMER (1923 b), PRZYLECKI (1924), HUF (1935 a, b, 1936 a, b, c) — showing that the isolated skin exposed to an identical solution (normal saline or Ringer) on both sides, does still transfer liquid through itself from the epithelial to the internal side, but that the amount is relatively small, was repeated ; as shown in Table V, normal (epithelium outside) and everted (epithelium inside) skins were filled with R_n and placed in aerated running R_n. The normal tube shows an increase in (Cl') and water ; the everted tube a fall in (Cl') and water. The observed fact was explained by those authorities with the exception of MAXWELL, upon the assumption that there is present a vital absorptive force. According to WERTHEIMER (1925), the transport of water is correlated with the difference of the ability of both surfaces to imbibe water. Though REID himself noticed it, JURIŠIĆ (1936) proposed the explanation against HUF (1935 a, b), that the swelling of the skin might make it appear that there is an appearance of inward transference of liquid. In view of the criticisms made by JURIŠIĆ, HUF (1936 c) re-
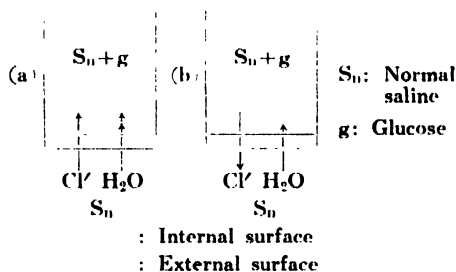
peated his experiment and confirmed the fact that while swelling reaches a maximum within several hours, absorption continues further. JURIŠIĆ (1936), obtained another result contrary to REID's and HUF's and sug- gested that HUF's data could not easily be reconciled with the view that the transport of fluid through the skin is due to vital activity and that if the transference exists, it might be due to something like the effect seen in JURIŠIĆ's previous experiment (1928): viz. when a gelatinized collodium membrane the one side of which has previously been in contact with acid and the other with alkali, both sides being afterwards washed, is spread between distilled waters, the water moves from the latter surface to the former. HUF (1936 c in " Nachtrag ") in answer to JURIŠIĆ attributed this opposite result to experimental technique. It may in my opinion, be explained as the result of the higher osmotic pressure on the internal side and within the membrane than on the epithelial side, which was caused by the active absorption of Cl' from without (epithelial side) in- wards and also partly by the hydration water of ions (11.4 mol H_2O for 1 mol NaCl) instead of the active absorption of the water.

2. Adaptation of the frog to isotonic sea-water and modification of asymmetry potential and of active absorption of Cl' through its isolated skin.

When frogs are put into salt solutions, salts are immediately absorbed through the skin into the body in hypertonic solutions in great quantities, in hypotonic in small quantities (ADOLPH 1927, 1933). This was the case with an isolated skin in my experiment, as previously mentioned. In a hypotonic solution the water initially gained begins to be eliminated after some time with the accumulated salt by the kidney and the normal water content of the frog is restored, while on the other hand in hypertonic salt solutions the water initially lost begins to be regained, until the body weight is greater than its initial value, and then begins to decrease to a new equilibrium value (REY 1938, see also ADOLPH 1933). The course of this recovery is fully accounted for by the rapid entrance of chloride, so that internal concentration becomes hypertonic with the outer solution (BRUNACCI 1912, DUVAL 1928, ADOLPH 1933, REY 1938). The later loss of weight is due to the dehydration, as the kidney cannot concentrate the urine above blood concentration (BRUNACCI 1912, TODA and TAGUCHI 1913, PRZYLECKI 1922, CRANE 1927, ADOLPH 1927, REY 1938, etc.), and,

therefore, must eliminate more water than is absorbed (REY 1938). But the dehydration causes the increase of internal concentration, which results in increase in water absorption and decrease in salt absorption. Then, after some time the absorbed solution will be isotonic with the blood, and the equilibrium is reached, which in effect cannot be attained unless the outer medium is somehow thinner than the blood. In a concentrated solution, frogs cannot survive long on account of the toxic action of the internal increase of concentration (REY 1938).

In each series of experiments, from 50 to 100 individuals were placed in R_x which was replaced by new R_x every day. The frogs that survived after some days were killed and their isolated skins were used for the determination of the active absorption of Cl' as previously done. As seen in Tables XIII, XIV, XV, XVI, XVII the asymmetry potential and the active absorption of Cl' was arrested or reduced, when the frogs were adapted to R_x; Table XVII indicates that when the same R_x was used on both sides, there was no change of concentration and no transfer of liquid, whether the normal or the everted skin was used. When the inner surface was exposed to R_x and the outer to $\frac{R_x}{2}$, Cl' was transferred through the isolated skin in the direction from the inner surface towards the

TABLE XIII.

Adaptation of Frog to R. and Modification of
active Absorption   Control   30 days in R_x
of Cl' through
isolated Skin.

Temp. $-20.0°C$

Outer solution $= \frac{R_x}{2}$ ∞ cc.

Inner solution $= R$, 2 cc.

---- : Internal surface   ——— : External surface

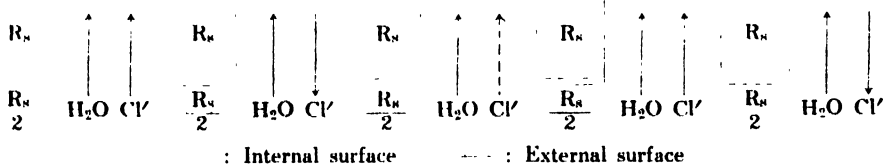| Frog No. Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq.	Water increased in inner sol.		Cl' increased in inner sol.		Surface exposed to outer sol.	Remarks
				cc.	%	m-eq.	%		
36+0 Dec. 20, 1937	1	5{ 10.44 a.m. 3.44 p.m.	0.1261	+0.050	+2.5	+0.0043	+1.7	External	Control Frog in tap-water I. S. =0.1271 N. Cl' O.S.=0.06355 N. Cl'
	2	5{ 4.01 p.m 9.01 ,,	0.1271	+0.050	+2.5	+0.0064	+2.5		
	3	10{ 9.24 p.m. 7.24 a.m.	0.1251	+0.097	+4.9	+0.0081	+3.2		

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	Water increased in inner sol. %	Cl' increased in inner sol. m-eq.	Cl' increased in inner sol. %	Surface exposed to outer sol.	Remarks
35	Dec 20, 1937	1	5 {11.04 a.m. / 4.04 p.m.}	0.1239	+0.045	+2.25	−0.0008	−0.3	External	Control Frog in tap-water I. S.=0.1271 N. Cl' O.S.=0.06355 N. Cl'
		2	5 {4.24 p.m. / 9.24 ..}	0.1236	+0.070	+3.30	+0.0017	+0.7		
		3	10 {9.56 p.m. / 7.56 a.m.}	0.1173	+0.185	+9.25	+0.0021	+0.8		
32	Dec. 16, 1937	1	5 {10.15 a.m. / 3.15 p.m.}	0.1246	+0.030	+1.50	−0.0013	−0.5	External	30 days in R$_k$ O.S.=0.1271 N. Cl' I. S.=0.0636 N. Cl' Skin potential Inital: 0.0025 V (No. 32) 0.0040 V (No. 33) Final: 0.0020 (No. 32) 0.0030 (No. 33)
		2	5 {3.33 p.m. / 8.33 ..}	0.1246	+0.035	+1.75	−0.0006	−0.2	External	
33		1	5 {10.28 a.m. / 3.28 p.m.}	0.1229	+0.052	+2.60	−0.0020	−0.8	Internal	
		2	5 {3.45 p.m. / 8.45 ..}	0.1226	+0.043	+2.20	−0.0037	−1.5	Internal	
49	Feb. 24, 1938	1	5 {10.38 a.m. / 3.38 p.m.}	0.1239	+0.040	+2.00	−0.0016	−0.6		30 days in R$_k$ I. S.=0.1271 N. Cl' O.S.=0.0636 N. Cl' Skin potential Inital: 0.0032 V (No. 49) 0.0017 V (No. 50) Final: 0.0090 0.0050 V (No. 49) 0.0015 0.0020 V (No. 50)
		2	5 {4.04 p.m. / 9.04 ..}	0.1250	+0.030	+1.50	−0.0005	−0.2		
		3	10 {9.22 p.m. / 7.22 a.m.}	0.1231	+0.055	+2.75	−0.0013	−0.5	External	
50		1	5 {11.05 a.m. / 4.05 p.m.}	0.1215	+0.085	+4.24	−0.0010	−0.4	External	
		2	5 {4 29 p.m. / 9.29 ..}	0.1233	+0.045	+2.25	−0.0022	−0.9		
		3	10 {9.51 p.m. / 7.51 a.m.}	0.12108	+0.070	+3.50	−0.0036	−1.4		

outer in accordance with the concentration gradient instead of the active absorption of Cl' in the reverse direction (Tables XIII, XIV). The intermediate case in which the active absorption was reduced to some extent, was also found according to the duration of the stay of the frogs in R$_k$ (XIV, XV, XVI). As the kidney of the frog is not capable of eliminating the urine in concentration above that of the blood, as previously described, the arrest of active absorption of Cl' seems to have some biological significance when frogs are placed in R., though this arrest may be due to narcosis etc.

TABLE XIV.

Adaptation of Frog to R. and Modification of active Absorption of Cl′ through isolated Skin.

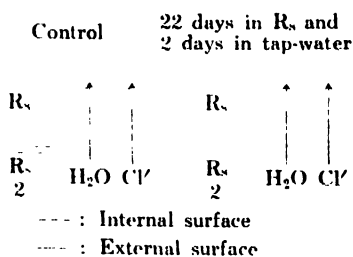Temp. $-20.0°C$; Outer solution $-\dfrac{R_{\text{м}}}{2}$ ∞ cc.; Inner solution $= R_{\text{к}}$ 2 cc.

Control	26 days in $R_\text{к}$	16 days in $R_\text{к}$	9 days in $R_\text{м}$

$$R_\text{м} \uparrow\uparrow \qquad R_\text{к} \downarrow\downarrow \qquad R_\text{м} \downarrow\uparrow \qquad R_\text{м} \downarrow\uparrow \qquad R_\text{м} \downarrow\downarrow$$

$$\frac{R_\text{к}}{2}\ H_2O\ Cl'\quad \frac{R_\text{к}}{2}\ H_2O\ Cl'\quad \frac{R_\text{м}}{2}\ H_2O\ Cl'\quad \frac{R_\text{к}}{2}\ H_2O\ Cl'\quad \frac{R_\text{к}}{2}\ H_2O\ Cl'$$

—— : Internal surface   — · — : External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl′ of inner sol. g.-eq. p. L.	Water increased in inner sol. cc.	Water increased in inner sol. %	Cl′ increased in inner sol. m-eq.	Cl′ increased in inner sol. %	Surface exposed to outer sol.	Remarks
♂→ 61	June 4, 1938	1	5 {10.48 a.m. / 3.48 p.m.	0.1232	+0.097	+4.9	+0.0040	+1.6	External	Control for No. 60 Frog in tap-water I. S.=0 1272 N. Cl′ O.S.=0.0636 N. Cl′
		2	5 {4.02 p.m. / 9.02 ,,	0.1237	+0.076	+3.8	+0.0024	+0.9		
♀ 60	June 4, 1938	1	5 {10.13 a.m. / 3.13 p.m.	0.1208	+0.058	+2.9	−0.0058	−2.2	External	26 days in $R_\text{к}$ healthy I. S.=0.1272 N. Cl′ O.S.=0.0636 N. Cl′
		2	5 {3.33 p.m. / 8.33 ,,	0.1224	+0.040	+2.0	−0.0048	−1.9		
♂→ 62	June 25, 1938	1	5 {10.48 a.m. / 3.48 p.m.	0.1251	+0.048	+2.4	+0.0020	+0.8	External	16 days in $R_\text{к}$ healthy I. S.=0.1271 N. Cl′ O.S.=0.0636 N. Cl′ Initial potential: 0.0007 V
		2	5 {4.04 p.m. / 9.04 ,,	0.1250	+0.033	+1.7	−0.001)	−0.0		
		3	10 {9.30 p.m. / 7.30 a.m.	0.1222	+0.064	+3.2	−0.0020	−0.8		
♂→ 63		1	5 {11.02 a.m. / 4.02 p.m.	0.1251	+0.040	+2.0	+0.0010	+0.4	External	16 days in $R_\text{к}$ healthy I. S.=0.1271 N. Cl′ O.S.=0.0636 N. Cl′ Initial potential: 0.0032 V
		2	5 {4.19 p.m. / 9.19 ,,	0.1256	+0.027	+1.4	+0.000(4)	+0.2)		
		3	10 {9.38 p.m. / 7.38 a.m.	0.1226	+0.065	+3.3	−0.0010	−0.4		
♂→ 41	Feb. 2, 1938	1	5 {11.00 a.m. / 4.00 p.m.	0.1249	+0.025	+1.25	−0.000(3)	−0.1)	External	9 days in $R_\text{к}$ I. S.=0.1266 N. Cl′ O.S.=0.0633 N. Cl′
		2	5 {4.28 p.m. / 9.28 ,,	0.1259	+0.045	+2.25	+0.0063	+2.5		
♀ 42		1	5 {11.16 a.m. / 4.16 p.m.	0.1201	+0.05	+2.5	−0.0070	−2.8	Internal	vigorous
		2	5 {4.38 p.m. / 9.38 ,,	0.1201	+0.06	+3.0	−0.0058	−2.3		

TABLE XV.

Adaptation and active Absorption.

Temp. $= 20.0°C$.

Outer solution $= \dfrac{R_n}{2}$ ∞ cc.

Inner solution $= R_n$ 2 cc.

Control   22 days in R_n and 2 days in tap-water

R_n R_n

$\dfrac{R_n}{2}$ H_2O Cl' $\dfrac{R_n}{2}$ H_2O Cl'

- - - : Internal surface

---- : External surface

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water inc. in inner sol. cc.	Water inc. in inner sol. %	Cl' increased in inner sol. m-eq.	Cl' increased in inner sol. %	Surface exposed to outer sol.	Remarks
65	July 21, 1938	1	5 {11.20 a.m. / 4.20 p.m.	0.1294	+0.072	+3.6	+0.0137	+5.4	External	Control Frog in tap-water I. S. $=0.1272$ N. Cl' Skin potential: Initial.... 0.051-0.0110 V Final....0.0155 V
		2	5 {4.40 ,, / 9.40 ,,	0.1272	+0.048	+2.4	+0.0061	+2.4		
		3	9 {9.54 ,, / 6.54 a.m.	0.1251	+0.090	+2.8	+0.0071	+2.8		
64		1	5 {10.10 a.m. / 3.10 p.m.	0.1267	+0.038	+1.9	+0.0038	+1.5		22 days in R_n and 2 days in tap-water I. S. $=0.1272$ N. Cl' Skin potential: Initial....0.0260 V Final....0.008-6 V
		2	5 {3.43 ,, / 8.43 ,,	0.1264	+0.045	+2.25	+0.0045	+1.6		
		3	9 {8.58 ,, / 5.58 a.m.	0.1250	+0.058	+2.9	+0.0029	+1.1		

TABLE XVI.

Adaptation, active Absorption and Skin Potential.

Outer solution $= \dfrac{R_n}{2}$ ∞ cc.;   Inner solution $= R_n$ 2 cc.;   Temp. $= 23.0°C$.

The outer surface was exposed to outer solution.

Frog No.	Date	Exp. No.	Time hrs	Final conc. of Cl' of inner sol. g.-eq. p. L.	Water inc. in inner sol. cc.	Water inc. in inner sol. %	Cl' increased in inner sol. m-eq.	Cl' increased in inner sol. %	Skin potential volt	Remarks
74	Sept. 10, 1938	1	4½ {0.15 p.m. / 4.45 ,,	0.1250	+0.055	+2.75	+0.0021	+0.8	0.0020 (11.03 a.m.) 0.0088 (4.50 p.m.)	32 days in R_n I. S. = 0.1274 N. Cl'
		2	4½ {5.30 ,, / 10.00 ,,	0.1250	+0.055	+2.75	+0.0021	+0.8	0.0051 (10.20 p.m.)	
75		1	4½ {0.35 p.m. / 5.05 ,,	0.1272	+0.050	+2.5	+0.0060	+2.4	0.0006 (11.55 a.m.)	31 days in R_n and 1 day in tap-water I. S. =
		2	4½ {5.40 p.m. / 10.10 ,,	0.1270	+0.025	+1.25	+0.0024	+0.9	0.0190 (10.30 p.m.)	0.1274 N. Cl'

Frog No	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g-eq. p. L.	Water inc. in inner sol. cc.	%	Cl' increased in inner sol. m-eq.	%	Skin potential volt	Remarks
♂ 76	Sept. 12, 1938	1	4½ {0.44 p.m. / 5.14 ,,}	0.1248	+0.055	+2.75	+0.0023	+0.9	0.008 (7.30 a.m.)	34 days in R_N and 5 h. 15 m. in tap-water I.S.= 0.1274 N. Cl'
		2	4½ {5.35 p.m. / 10.05 ,,}	0.1245	+0.050	+2.5	+0.0009	+0.4	0.010 (10.15 p.m.)	
♂ 77	Sept. 14, 1938	1	4 {1.55 p.m. / 5.55 ,,}	0.1255	+0.032	+1.6	+0.0010	+0.4	0.0083 (7.30 a.m.) ↓ both = R_N 0.0098 (8.00 a.m.) 5h10m I.S.=R_N O.S.=T	36 days in R_N I.S.= · 0.1270 N. Cl'
		2	4 {6.32 ,, / 10.32 ,,}	0.1250	+0.040	+2.0	+0.0009	+0.4	0.0044↓ (1.10 p.m.)	
		3	10 {10.53 ,, / 8.53 a.m.}	0.1224	+0.060	+3.0	−0.0018	−0.7	0.0050 (6.17 p.m.) 0.0000 (9.15 a.m., Sept. 15)	

TABLE XVII.

Adaptation to R_N and Abolition of active Absorption.

Inner solution = R_N 2 cc.; Outer solution = R_N ∞ cc.; Temp. = 20.0°C.

Forg No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g-eq. p. L	Water inc. in inner sol. cc.	%	Cl' increased in inner sol. m-eq.	%	Surface exposed to outer sol.	Skin potential volt	Condition of the frog	Remarks
→ 29	Dec. 9, 1937	1	5 {11.34 a.m. / 4.34 p.m.}	0.1332	0	0	0	0	Internal	Initial: 0.0043	not vigorous	23 days in R_N
		2	5 {5.01 ,, / 10.01 ,,}	0.1332	0	0	0	0				
♀ 30		1	5 {0.01 p.m. / 5.01 ,,}	0.1332	0	0	0	0	External	Initial: 0.0032	vigorous	R_N = 0.1332 N. Cl'
		2	5 {5.17 ,, / 10.17 ,,}	0.1332	0	0	0	0				

When however the frogs, adapted to R_N, were returned to the tap water, or their outer surface was in contact with the tap water and the inner with R_N, the active absorption of Cl' and the skin potential were restored more or less (compare Table XVIII with Table IX and see Tables XV, XVI).

TABLE   XVIII.

Adaptation to R, and Modification of active Absorption and of Skin Potential.

Temp. =20.0°C; Inner solution = R_s 2 cc.

Outer solution = Tap-water (ca. 0.001 N. Cl') ∞ cc.

The external surface was exposed to the outer solution.

Frog No.	Date	Exp. No.	Time hrs.	Final conc. of Cl' in inner sol. g-eq. p. L.	Water inc. in inner sol.		Cl' increased in inner sol.		Skin potential volt	Remarks
					cc.	%	m-eq.	%		
31	Dec. 14, 1937	1	5{10.24 a.m. / 3.24 p.m.	0.1216	+0.065	+3.25	−0.0031	−1.2	Initial: 0.0045	23 days in R. I.S. = 0.1271 N. Cl' Collection: Nov. 11
		2	5{3.42 p.m. / 8.42 ,,	0.1226	+0.060	+3.0	−0.0016	−0.6	Final: 0.0020	
45	Feb. 15, 1938	1	5{8.38 a.m. / 1.38 p.m.	0.1234	+0.055	+2.75	−0.000(3)	−0.(1)		
		2	5{1.54 p.m. / 6.54 ,,	0.1230	+0.060	+3.0	−0.000(4)	−0.2	Initial: 0.0100	
		3	5{7.08 p.m. / 0.08 a.m.	0.1231	+0.065	+3.25	−0.000(4)	+0.2		10 days in R. I.S. = 0.1269 N. Cl'
46		1	5{8.53 a.m. / 1.53 p.m.	0.1223	+0.085	+4.25	+0.0012	+0.5		
		2	5{2.10 p.m. / 7.10 ,,	0.1233	+0.060	+3.0	+0.000(1)	+0.0	Initial: 0.0442	
		3	5{7.24 p.m. / 0.24 a.m.	0.1234	+0.060	+3.0	+0.0004	+0.2		
47	Feb. 23, 1938	1	5{10.55 a.m. / 3.55 p.m.	0.1221	+0.075	+3.75	−0.0009	−0.4		
		2	5{4.12 p.m. / 9.12 ,,	0.1227	+0.06	+3.0	−0.0014	−0.6	Initial: 0.0190	
		3	10{9.34 p.m. / 7.34 a.m.	0.1180	+0.105	+5.25	−0.0058	−2.3		29 days in R. I.S. = 0.1271 N. Cl'
48		1	5{11.25 a.m. / 4.25 p.m.	0.1208	+0.08	+4.0	−0.0030	−1.2		
		2	5{4.41 p.m. / 9.41 ,,	0.1216	+0.06	+3.0	−0.0037	−1.5	Initial: 0.0027	
		3	10{10.02 p.m. / 8.02 a.m.	0.1175	+0.095	+4.75	−0.0080	−3.2		
51	April 7, 1938	1	5{11.40 a.m. / 4.40 p.m.	0.1195	+0.10	+5.0	−0.0027	−1.1	Initial: 0.0023	25 days in R. vigorous I.S. = 0.1268 N. Cl'
		2	5{5.07 p.m. / 10.07 ,,	0.1208	+0.085	+4.25	−0.0019	−0.8	Final: 0.0000	
		3	10½{10.33 p.m. / 9.03 a.m.	0.1139	+0.13	+6.5	−0.0110	−4.3		

Frog No. / Date / Exp. No.	Time hrs.	Final conc. of Cl' of inner sol. g-eq. p. L.	Water inc. in inner sol. cc.	%	Cl' increased in inner sol. m-eq.	%	Skin potential volt	Remarks
♀ 52 April 7, 1938 — 1	5 {11.53 a.m. / 4.53 p.m.}	0.1177	+0.135	+6.75	−0.0023	−0.9	Initial: 0.0016 Final: 0.0028	25 days in R. vigorous I. S. = 0.1268 N. Cl'
2	5 {5.22 p.m. / 10.22 ,,}	0.1215	+0.070	+3.5	−0.0021	−0.8		
3	10½ {10.43 p.m. / 9.13 a.m.}	0.1157	+0.133	+6.65	−0.0069	−2.7		
♂ 53 April 22, 1938 — 1	5 {0.18 p.m. / 5.18 ,,}	0.1211	+0.072	+3.6	−0.0023	−0.9	Initial: 0.0325 Final: 0.0225	32 days in R. and thereafter 13 days in tap-water I. S. = 0.1268 N. Cl' vigorous
2	5 {5.40 p.m. / 10.40 ,,}	0.1217	+0.067	+3.35	−0.0021	−0.8		
3	10 {11.00 p.m. / 9.00 a.m.}	0.1164	+0.134	+6.7	−0.0053	−2.1		
♀ 54 May 24, 1938 — 1	5 {8.27 a.m. / 1.27 p.m.}	0.1210	+0.067	+3.35	−0.0031	−1.2	Initial: 0.0120 Final: 0.0080	56 days in R. and thereafter 16 days in tap-water and died I. S. 0.1266 N. Cl'

3. Survival of the frog in isotonic sea-water.

As regards the duration of the survival of the frogs in diluted sea-water, BERTIN (1920) found that *Rana temporaria* could not tolerate concentration above 7‰ for more than 24 hours, but survived long in 3‰, while DUVAL (1928) observed that it was sound after 24 hours in 9.7‰ (J: −0.61) and lived for many days in 8.5‰ (J: −0.51). According to REY (1938) the survival of *Rana esculenta* did not exceed 4 days in diluted sea-water higher than or equal to one half of the concentration of blood, viz. $\frac{1}{10}$ of concentration of sea-water; in $\frac{1}{20} - \frac{1}{50}$ of the concentration it could, sometimes survive for 15 days. These differences in survival, in REY's opinion, may be due to the toxic salts which were absorbed at the beginning of the stay of the frog in sea-water.

In my experiments from 50 to 100 frogs from the same source were placed in isotonic sea-water, which was renewed daily. The temperature of the solution was not controlled and varried with room temperature. In this condition some frogs swelled owing to increase of subcutaneous lymph (see REY 1938) while others did not swell. The half of the frogs died within from 10 to 15 days and the other half within from about 25 to

40 days. When plotted with respect to day, the number of the dead frogs is maximal near on the 10th–15th day from the beginning of the experiment and gradually decreases towards the both sides (Table XIX).

TABLE XIX.

Numbers of dead Frogs in isotonic Sea-water.

Day after immersion / Date	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Jan. 24, '38→	0	0	0	0	2	1	1	1	1	1	3	11	9	3	1	4	3	4	4
May 9, '38→	0	0	1	6	3	1	10	5	8	15	9	12	12	3	4	4	2	0	1
Aug. 8, '38→	0	2	1	5	7	4	6	5	3	1	7	10	7	6	7	3	3	7	1
Sept. 23, '38→	0	1	2	3	0	1	1	5	1	10	24	20	4	3	10	3	0	2	7

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
5	3	3	3	3	1	0	3															
1	3	1	0	1	1	1	1	0	0													
1	0	0	1	0	0	0	0	0	0	0	1	3	2	1	0	0	0	0	0			
1	0	0	1	0	0	0	1															

→ indicates the day at which half of the frogs died.

4. The potential and respiration of the isolated skin and their modification in the adaptation of the frog to isotonic sea-water.

As shown in Table XX, the adaptation of the frog to R$_x$ for about a month reduced both the O$_2$-consumption and the potential of the isolated skin, but not to the same extent; while the potential was reduced to a few millivolts from about 30 to 110 millivolts, O$_2$-consumption was maintained at 80% of its normal value. LUND (1928) and others found that both O$_2$-consumption and potential were reduced when O$_2$-tension was diminished, although not in the same manner. While FRANCIS (1934) found that the potential and respiration depend in the same way on O$_2$ concentration, TAYLOR (1935) observed that the lowering of the potential is usually about 20% greater than the lowering in the O$_2$-consumption,

TABLE XX.

Oxygen Consumption of isolated Skin in isotonic Sea-water.

O_2-consumption was measured in 30 cc. of isotonic sea-water.
April 20, 1938. Temp. = 20.0°C.

Weight of skin		O_2 in 30 cc. of solution cc.	O_2-consumption					Remarks
Wet gr.	Dry gr.		cc.	cc./gr. wet wt./hr.	*	cc./gr. dry wt./hr.	*	
0.1162	0.0294	0.148	0.0113	0.097	100	0.38	100	Control Frog, 39 days in tap-water since collection
0.1506	0.0401	0.155	0.0125	0.083	86	0.31	82	Frog, 37 days in R₈
0.1202	0.0332	0.143	0.0113	0.094	97	0.34	90	Frog, 29 days in R₈ and 10 days in tap-water

* relative value (control = 100)

when the skin is exposed to N_2/O_2 or CO/O_2 mixtures. PONDER and MACLEOD (1936) observed, in the study of the effect of the homologous carbamates, that the potential was reduced to zero, for example, in a concentration of ethyl carbamate which reduced the respiration to 50% only. The simple lysins (saponin and the bile salts), on the other hand, abolish the potential without reducing the O_2-consumption at all. As maintained by PONDER and MACLEOD, the frog skin is a heterogeneous tissue, and the potential difference may arise in a spatially defined part of the skin. When one measures the O_2-consumption of the whole skin, one is not necessarily measuring the O_2-consumption in the regions which determine the potential difference. According to the calculation of FRANCIS (1933), the output of electrical energy is several per cent of the oxidation energy of the frog skin. As previously stated, a similar relationship was observed by RUBINSTEIN and PEVSNER (1937) between the potential and the irreciprocal permeability as exemplified by the skin of a frog, whose potential was reversibly or irreversibly abolished in an isotonic KCl solution, or in distilled water, while its irreciprocal permeability to methylen blue was maintained.

5. Adaptation of the frog to distilled water and modification of active absorption of Cl' through the isolated skin.

KROGH (1937 a, b) has shown that in a number of fresh-water animals osmotic regulation is largely brought about by a process of active absorp-

tion from the very diluted solutions represented by the fresh-water, or from equally diluted solutions prepared artificially, and that this absorption can as a rule be demonstrated only when the store of salt in the organism is depleted by washing with distilled water; the mechanism of this absorption was studied by KROGH (1938). According to him, the intact frog sprayed for some time with distilled water began to absorb Cl′ through the skin from so diluted a solution as $\frac{Ringer}{100}$.

As seen in Table XXI, the isolated skin of frogs which was placed in distilled water for some days, seemed to indicate that there is an active absorption of Cl′ from $\frac{R_\kappa}{100}$ in the direction from the epithelial to the internal side, while that which was placed in tap water lost Cl′ to $\frac{R_\kappa}{100}$, though only slightly.

<div align="center">TABLE XXI.</div>

Adaptation of Frog to distilled Water and increase of active Absorption of Cl′ through isolated Skin of Frog.

Outer solution $= \frac{R_\kappa}{100}$; Inner solution $= R_\kappa$; Temp. $= 20.0°C$.

The outer surface was exposed to outer solution.

Frog No. Date Exp. No.			Time hrs.	Final conc. of Cl′ of inner sol. g-eq. p. L.	Water increased in inner solution cc.	Water increased in inner solution %	Cl′ increased in inner solution m-eq.	Cl′ increased in inner solution %	Remarks
78 Sept. 27, 1938	1	5	10.06 a.m. / 3.06 p.m.	0.1230	+0.055	+2.75	+0.0001	+0.0	Frog, freshly caught I. S. = 0.1263 N. Cl′ O.S. = 0.001263 ,,
	2	5	3.25 ,, / 8.25 ,,	0.1233	+0.055	+2.75	+0.0001	+0.0	
79	1	5	10.29 a.m. / 3.29 p.m.	0.1205	+0.090	+4.5	−0.0010	−0.4	
	2	5	3.50 ,, / 8.50 ,,	0.1212	+0.085	+4.25	−0.0007	−0.3	
80 Oct. 11, 1938	1	5	10.45 a.m. / 3.45 p.m.	0.1225	+0.088	+4.4	+0.0031	+1.2	Frog, 14 days in distilled water I. S. = 0.1263 N. Cl′
	2	5	4.05 ,, / 9.05 ,,	0.1218	+0.073	+3.65	−0.0002	0.1	
81	1	5	10.55 a.m. / 3.55 p.m.	0.1208	+0.085	+4.25	−0.0008	−0.3	Control Frog, 14 days in tap-water (ca. 0.001 N. Cl′) I. S. = 0.1263 N. Cl′
	2	5	4.10 ,, / 9.10 ,,	0.1205	+0.078	+3.6	−0.0023	−0.9	

Frog No. / Date	Exp. No	Time hrs.	Final conc. of Cl′ of inner sol. g-eq. p. L.	Water increased in inner solution cc.	Water increased in inner solution %	Cl′ increased in inner solution m-eq.	Cl′ increased in inner solution %	Remarks
+83 Oct. 12, 1938	1	5 {10.10 a.m. / 3.10 p.m.	0.1200	+0.108	+5.4	+0.0002	+0.1	Frog, 15 days in distilled water
	2	5 { 3.27 ,, / 8.27 ,,	0.1222	+0.091	+4.6	+0.0029	+1.1	I. S.=0.1263 N. Cl′
+82 Oct. 12, 1938	1	5 {10.00 a.m. / 3.00 p.m.	0.1209	+0.080	+4.0	−0.0012	−0.5	Control Frog, 15 days in tap-water
	2	5 { 3.20 ,, / 8.20 ,,	0.1212	+0.080	+4.0	−0.0007	−0.3	I. S.=0.1263 N. Cl′
♂ 96 Oct. 24, 1938	1	5 {10.13 a.m. / 3.13 p.m.	0.1190	+0.150	+7.5	+0.0004	+0.2	Frog, 27 days in distilled water
	2	5 { 3.39 ,, / 8.39 ,,	0.1198	+0.133	+6.7	+0.0001	+0.0	I. S.=0.12774 N. Cl′
+97 Oct. 24, 1938	1	5 {10.26 a.m. / 3.26 p.m.	0.1218	+0.070	+3.5	−0.0033	−1.3	Control Frog, 27 days in tap-water
	2	5 { 3.46 ,, / 8.46 ,,	0.1225	+0.054	+2.7	−0.0039	−1.5	I. S.=0.12774 N. Cl′

IV. SUMMARY

1. In isotonic sea-water the skin potential of the frog was maintained longer than in frog Ringer.

2. When isotonic sea-water (R_n) was exposed to the internal side of the isolated skin of the frog and various concentrations from $\frac{R_n}{10}$ to 2 R_n to the external (epithelial) side, there was an active absorption of Cl′ from the external to the internal side, even in resistance to osmotic pressure. This process takes place in great quantities in a high concentration and in small quantities in a diluted solution. Into tap water or $\frac{R_n}{100}$, outward Cl′-diffusion was practically O or very slight.

3. Passage of water through the isolated skin in physiological conditions seemed to be controlled by no force but osmotic pressure.

4. REID's findings that the fresh, isolated skin, exposed to the same normal saline on both sides, does still transfer liquid through itself from the external to the internal side, are considered not to be explained by a vital absorptive force as many authorities have assumed, but by (1) the higher osmotic pressure in the solution, facing the internal side and within the skin than that exposed to the external side, which is caused

by the active absorption of Cl' from the epithelial to the internal side and partly by (2) hydration water of absorbed ions.

5. Active absorption of Cl' was arrested or reduced when frogs were placed in the isotonic sea-water for about a month; that is when the internal side of the isolated skin faced R_s and the external side $R_s/2$, Cl' was not actively absorbed through itself in the direction from the epithelial to the internal side, but was transferred in the inverse direction in accordance with osmotic gradient.   When R_s was used on both sides, there was no transport of Cl' and water. The skin potential was also reduced.

6. When however the frogs, adapted to R, were returned to the tap-water or the epithelial side of the isolated skin of the adapted frog was exposed to tap-water and the internal side to R_s, then the active absorption of Cl' and the skin potential were restored more or less.

7. Survival of frogs in R_s ($\varDelta$: -0.46) for a relatively long time was observed.

8. Long adaptation of frogs to R_s reduced the potential of the isolated skin to a few m.v. from 30-110 m.v. while the O_2-consumption of it was maintained at 80% of its normal value.

9. The isolated skin of the frogs adapted to distilled water indicated active absorption of Cl' from $\frac{R_s}{100}$.

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STUDIES ON THE EXCHANGE AND THE EQUILIBRIUM OF WATER AND ELECTROLYTES IN A HOLOTHURIAN, *CAUDINA CHILENSIS* (J. MÜLLER)*

VI. ON THE NON-EXISTENCE OF THE ASYMMETRY POTENTIAL AND IRRECIPROCAL PERMEABILITY OF THE ISOLATED BODY-WALL TO CHLORIDE ION AND WATER

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The irreciprocal permeability of the surface of the aquatic animals (absorption or secretion) has been considered to be correlated with their osmo-regulatory power (see SCHLIEPER 1935). As *Caudina* is a poikil-osmotic animal (KOIZUMI 1933), the irreciprocal permeability of the body wall to salts and water is non-existent (KOIZUMI 1933, 1935 a, b). The present investigation is concerned with a further proof of this fact, an identical and isolated body wall being used in order to avoid the effects of individual differences in the specimens.

MATERIAL AND TECHNIQUE

Caudina obtained at Asamushi was used. It was for one day kept in the diluted sea-water which was to be used for the inner medium in the experiment, and then in mentholed and diluted sea-water of the same concentration for one more day in order to obtain its non-contracted body-wall as the membrane. The animal was cut longitudinally with scissors, and, after the removal of its entire viscera, was well washed with the same mentholed sea-water. Then the desired size of the wall was wiped with filter paper and spread in an uncontracted condition over the lower end of the graduated tube.

The apparatuses and details of the experimental procedure are the same as those given in a previous paper (KOIZUMI 1938). 2 cc. of a solution were measured accurately into the tube with the body-wall, and

after the meniscus had been read, the tube was kept under running diluted sea-water for half an hour or more in order to remove the effect of the anaesthetic. Before making the experiment the preparation was kept in the same experimental condition for from 10 to 30 minutes. The determination of chloride ion and potential was made as done previously (KOIZUMI 1938).

RESULTS AND DISCUSSION

As shown in Tables 1 and II, the rate of permeation of chloride ion, and water through the isolated body wall of *Caudina* is constant, in a direction either from without inwards or from within outwards, throughout the experimental course, viz. at least for 10 hours in the identical body wall.

TABLE I.

Caudina chilensis, one day in Sea-water (Cl' = 16.54 g/L).

Sep. 29, 1937; Temp. = 20.0°C.
Inner solution = Sea-water (Cl' = 16.54 g/L) 2 cc.
Outer solution = dil. Sea-water (Cl' = 12.04 g/L) ∞ cc.

Caudina No.	Exp. No.	Time hrs.	Final conc. of Cl' of inner solution g/L	Water increased in inner sol. cc.	%	Cl' increased in inner sol. mg.	%	Surface exposed to outer solution	Remarks
I	1	2.5 {10.00 a.m. / 0.30 p.m.}	16.03	+0.030	+1.5	−0.54	−1.63		
	2	2.5 {0.45 p.m. / 3.15 ,,}	16.03	+0.030	+1.5	−0.54	−1.63		
	3	2.5 {3.45 p.m. / 6.13 ,,}	15.98	+0.035	+1.8	−0.56	−1.69	External	
	4	2.5 {6.34 p.m. / 9.04 ,,}	15.98	+0.030	+1.5	−0.64	−1.93		
II	1	2.5 {10.17 a.m. / 0.47 p.m.}	15.88	+0.020	+1.0	−1.00	−3.02		
	2	2.5 {1.05 p.m. / 3.35 ,,}	15.88	+0.025	+1.3	−0.92	−2.78		
	3	2.5 {3.50 p.m. / 6.20 ,,}	15.88	+0.015	+0.75	−1.08	−3.26	Internal	
	4	2 5 {6.41 p.m. / 9.11 ,,}	15.88	+0.015	+0.75	−1.08	−3.26		

*The arrangement of longitudinal muscles on the skin spread over the lower end of the glass tube.

TABLE II.

Caudina chilensis, one day in Sea-water $(Cl' = 16.54 \, g/L)$.

Sep. 27, 1937; Temp. $=20.0°C$.
Inner solution $=$ Sea-water $(Cl' = 16.54 \, g/L)$ 2 cc.
Outer solution $=$ Concentrated Sea-water $(Cl' = 21.04 \, g/L)$ ∞ cc.

Caudina No.	Exp. No.	Time hrs.	Final conc. of Cl' of inner solution g/L	Water increased in inner sol.		Cl' increased in inner sol.		Surface exposed to outer solution	Remarks
				cc.	%	mg.	%		
I	1	2.5 { 9.48 a.m. / 0.18 p.m.	17.50	−0.015	−0.75	+1.66	+5.02		
	2	2.5 { 0.34 p.m. / 3.04 „	17.55	−0.010	−0.50	+1.84	+5.56		*
	3	2.5 { 3.30 p.m. / 6.00 „	17.46	−0.020	−1.0	+1.49	+4.50	External	
	4	2.5 { 6.16 p.m. / 8.46 „	17.50	−0.020	−1.0	+1.57	+4.75		
II	1	2.5 { 10.03 a.m. / 0.33 p.m.	17.23	−0.025	−1.3	+0.95	+2.87		
	2	2.5 { 0.45 p.m. / 3.15 „	17.19	−0.027	−1.4	+0.84	+2.54		*
	3	2.5 { 3.39 p.m. / 6.09 „	17.23	−0.028	−1.4	+0.90	+2.72	Internal	
	4	2.5 { 6.29 p.m. / 8.59 „	17.28	−0.025	−1.3	+1.05	+3.17		

* The arrangement of longitudinal muscles on the skin spread over the lower end of the glass tube.

If the direction of the osmotic force through the body wall, within its physiological conditions, is changed without alteration of the magnitude at regular intervals, the rate of permeation in the direction from within outwards will be comparable with that in the reverse direction in the identical body wall. That is, while sea-water of 16.54 g. Cl' per L. was used as the inner solution, sea-water of 21.04 g. Cl' per L. or that of 12.04 g. Cl' per L. was used alternately as the outer solution at intervals of $2\frac{1}{2}$ hours, since $21.04 - 16.54 = 16.54 - 12.04$, and the osmotic pressure of sea-water is practically proportional to a Cl' concentration of it (see KOIZUMI 1938, and Tabulae Biologicae IV 1927).

The results are stated in Table III, which indicates that the irreciprocal permeability of the isolated body-wall to Cl' and water is non-existent in *Caudina*, whether the orientation of the body-wall, which was spread over the graduated tube, is normal (its physiologically internal surface

TABLE III.

Caudina chilensis, one day in Sea-water $(Cl' = 16.54\ g/L)$.

Sep. 25, 1937; Temp. = 20.0°C.
Inner solution = Sea-water $(Cl' = 16.54\ g/l.)$ 2 cc.
Outer solution = dil. Sea-water* ∞ cc.

Caudina No.	Exp. No.	Time hrs.	*Concentration of Cl' of outer sol. g/L	Final conc. of Cl' of inner solution g/L	Water inc. in inner sol. cc.	%	Cl' increased in inner sol. mg.	%	Surface exposed to outer sol.	Remarks
I	1	2.5 { 0.00 p.m. / 2.30 „	12.04	16.03	+0.020	+1.0	-0.70	-2.1	External	*
	2	2.5 { 3.14 p.m. / 5.44 „	21.04	17.12	-0.017	-0.85	+0.87	+2.6		
	3	2.5 { 6.21 p.m. / 8.51 „	12.04	16.03	+0.020	+1.0	-0.70	-2.1		
	4	2.5 { 9.13 p.m. / 11.43 „	21.04	17.10	-0.015	-0.75	+0.86	+2.6		
II	1	2.5 { 0.30 p.m. / 3.00 „	12.04	15.89	+0.030	+1.5	-0.81	-2.5	Internal	*
	2	2.5 { 3.28 p.m. / 5.58 „	21.04	17.32	-0.030	-1.5	+1.04	+3.1		
	3	2.5 { 6.29 p.m. / 8.59 „	12.04	15.76	+0.022	+1.1	-1.21	-3.7		
	4	2.5 { 9.22 p.m. / 11.52 „	21.04	17.55	-0.030	-1.5	+1.49	+4.5		

* The arrangement of longitudinal muscles on the skin spread over the lower end of the glass tube.

being faced toward the inner solution) or reversed.

As seen in Table IV the skin potential was found to be 0, when the leads were symmetric, i. e., when both sides of the body wall were exposed to identical sea-water.

TABLE IV.

July 13, 1936. Temp. = 20.0°C.

Time mins.	Skin Potential m.v.	Remarks
0	0.0	Killed with or without anaesthetic.
18	0.0	Inner Solution = Outer solution:
75	0.0	Sea-water.
135	0.0	Orientation of the Skin: normal or
180	0.0	everted.

The absorption or excretion (irreciprocal permeability) has sometimes been considered in connection with asymmetric properties, especially with the asymmetry potential (MOND 1927, AMSON 1930, KELLER 1931, LEUT-HARDT and ZELLER 1934, HUF 1935, RUBINSTEIN and PEVSNER 1937, WILBRANDT 1938 etc.). As the body wall of *Caudina* has no such gland-like activity to salts, it may be expected that when the leads are symmetric, the asymmetry potential does not exist.

SUMMARY

1. The absence of the so-called "*irreciprocal permeability*" of the body wall of *Caudina chilensis* to Cl' and water was re-proved.

2. The potential difference across the body wall was found to be 0, when both sides of it were exposed to identical solution (sea-water).

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ON CORPUSCLES IN THE BODY FLUIDS OF SOME INVERTEBRATES

GENERAL CONSIDERATIONS ON THE RESULTS OBTAINED BY THE PRECEDING INVESTIGATION[1]

BY

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A survey of the literature on the subject reveals the existence of very many classic works treating on the blood cells of invertebrates. Most of them, however, are unsatisfactory from the point of the investigation techniques. Relatively recently KIYONO and his students made researches on the haemolymph of various invertebrates, in the main using the methods of vital and supravital staining. The results of these investigations are recorded in detail in his monograph on vital staining (KIYONO 1929). In this monograph, however, the exact species examined are not definitely indicated. JORDAN (1933) gave a brief description of the evolution of metazoan blood. But in so far as I am aware these two papers are the only literature dealing with the phylogenetic relation of invertebrate blood corpuscles. I have published also a series of reports on the coelomic or blood corpuscles of various invertebrates. Since this series of reports was made only on animals which the writer at hand, there are only a few systematic descriptions which may suggest the characteristics of invertebrate blood. This causes various inconveniences to the promotion of further investigation. Accordingly I wish here to summarize the results obtained by the previous observations. Such a summary is believed to be worthy of publication, as it may help to throw some light upon invertebrate haematology which still remains obscure in many details.

The animals utilized in my work are as follows:

Echinoderma.

Holothuroidea: *Leptosynapta in haerens* (O. F. MÜLLER), *Caudina chilensis* (J. MÜLLER), *Molpadia roretzii* (V. MARENZELLER).

Asteroidea: *Henrica leviuscula* var. *nipponica* UCHIDA, *Aphelasterias japonica* (BELL), *Asterina pectinifera* (MÜLLER et TROSCHEL).

Echinoidea: *Temnopleurus hardwickii* (GRAY).

Annelida.

Chaetopoda :
  a) Polychaeta : *Euphrosyne superba* MARENZELLER, *Ceratocephale osa-wai* IZUKA, *Nereis mictodonta* MARENZELLER, *Glycera chirori* IZUKA, *Travisia japonica* FUJIWARA, *Potamilla torelli* MALMGREN, *Terebella* sp. (*debilis ?*).
  b) Oligochaeta : *Drawida hattamimizu* HATAI, *Pheretima sieboldi* (HORST), *Ph. hilgendorfi* (MICHAELSEN).
Echiuroidea : *Urechis unicinctus* (VON DRASCHE), *Thalassema gogoshimense* IKEDA.
Sipunculoidea : *Physcosoma scolops* SELENKA et DE MAN, *Sipunculus nudus* LINNÉ, *Siphonosoma cumanense* (KEFERSTEIN).

Molluscoidea.

Brachiopoda : *Terebratalia coreanica* ADAMS et REEVE, *Coptothyris grayi* (DAVIDSON), *Lingula unguis* LINNÉ.

Mollusca.

Gastropoda : *Cuthona (Cuthona) bicolor* BERGH.
Lamellibranchia : *Ostrea nippona* SEKI, *Mytilus grayanus* DUNKER, *M. edulis* LINNÉ, *Anomia lisckei* DAUTZENBERG et FISCHER, *Libitina japonica* (PILSBRY). *Glycimeris vestitus* DUNKER, *Arca inflata* (REEVE).
Cephalopoda : *Idiosepius paradoxa* (ORTMANN), *Octopus dofleini* (WÜLKER).

Arthropoda.

Crustacea : *Tachypleus tridentatus* LEACH, *Brachionotus sanguineus* (DE HAAN), *Huenia proteus* DE HAAN, *Eriocheir japonica* DE HAN, *Spirontocaris pandaloides* (STIMPSON).

Prochordata.

Tunicata : *Cynthia roretzi* V. DRASCHE, *Styela clava* HERDMAN, *Chelyosoma siboja* OKA, *Corella japonica* var. *asamushi* OKA.

There are many other kinds of animals to be studied, such as Trematoda, Nemertini, Insecta, Arachnoidea, and others, of which future investigations will treat.

The formed elements in the body fluid of invertebrates may be distinguished as follows: A. Erythrocytes; B. Leucocytes; a) hyaline leucocytes or lymphocytes, b) granular leucocytes, c) pigmented leucocytes and

d) specially formed elements. I frequently used the term 'lymphocyte' in my previous papers. But this word is meaningless when one recalls that no difference of myeloid and lymphatic origin of cells is discernible in invertebrates. Therefore, 'lymphocyte' is the name we have given to the leucocytes which have hyaline, colourless cytoplasm, and which are active to amoeboid movement, phagocytosis and thrombogenesis. These so-called lymphocytes show practically no difference in structure and function as compared with the corresponding cells of vertebrates. KIYONO (1929) usually classified the haemolymph cells by their size, and he purposely avoided applying the classification of vertebrate blood-corpuscles to that of haemolymph cells. However I incline to use the terms of vertebrate haematology, because, as will soon be seen, it seems that no real gap exists between the blood cells of vertebrates and those of invertebrates. Furthermore to unify the classification is convenient in order to compare the blood cells of both groups of animals.

A. ERYTHROCYTES

KRUMBHAAR stated in the 'Special Cytology' edited by COWDRY (1932) that no other cell in the mammalian body has been studied so extensively as the red corpuscles of the blood. The situation, however, is just the reverse in so far as invertebrate erythrocytes is concerned.

In the animals utilized for the present investigation, red coloured cells of the blood were found in 13 species; *Physcosoma scolops, Sipunculus nudus, Siphonosoma cumanense, Urechis unicinctus, Thalassema gogoshimense, Molpadia roretzii, Caudina chilensis, Travisia japonica, Glycera chirori, Terebella* sp., *Lingula unguis, Arca inflata,* and *Glycimeris vestitus.* It is well known that the first three species possess red cells which contain haemerythrin while the others have red cells with haemoglobin.

Shape: The shape of the red cells is circular, oval or elliptic in outline, and they are biconcave or biconvex disks, with smooth contour and round edges. The shape, however, shows a good deal of variation, owing to the change of external conditions. The red cells of *Molpadia* and *Caudina* frequently take a spindle or cocoon shape, with one, two, or three long, spine-like protuberances in vitro (Fig. 1, OHUYE, 1936 b). Red cells in the shapes of cap, trigon, tetragon, etc. are encountered in the blood of *Physcosoma* (Fig. 5, OHUYE, 1937 a). The red cells of *Thalassema* always assume irregular shapes with lobular pseudopodia-like protuberances from the cell surface (Fig. 4, OHUYE, 1937 a). The hyper-

tonic solution, needless to say, causes the shrinking of red cells while the hypotonic solution acts to increase the cell volume (OHUYE, 1936 b, '37 a).

It is well known that relatively uniform shapes (circular or elliptical in outline) are met with in the red cells of the vertebrates, and that they do not easily change their shape in the course of microscopic examination, if the preparation has been made carefully. But the red cells of invertebrates were found to be easily deformed in vitro, despite the fact that the observation was made immediately after the withdrawal of the body fluid (OHUYE, 1936 b, '37 a).

Size: The accurate determination of the average size of the red cells was not included in the present investigation, for these cells change their shape very easily on the outside of the animal body, as was mentioned just above. The measurement of their diameters was always made with the red cells suspended in the body fluid. The size of red cells shows a considerable variation in different animals. The smallest is the red cell of *Siphonosoma* (OHUYE, 1938 b) which measures 10 μ in diameter, and 3 μ in thickness, while the largest is that of *Urechis* (OHUYE, 1937 a), measuring 25 μ across and 4 μ in thickness. In the present observation, therefore, there was found no cell so large as the red cells of an amphibian, *Proteus* (70 $\times$ 41 μ) nor any so small as of a mammal, *Moschus* (2.5 across). In other words, the size of invertebrate erythrocytes shows a narrower fluctuation than that of vertebrate erythrocytes. The thickness of red cells is not always parallel to their diameter. The thinnest is the red cell of *Arca* (OHUYE, 1937 a), the thickness of which is only about 1.5 μ while the diameter reaches approximately 20 μ.

Nucleus: Needless to say, the red cells of invertebrates are nucleated in principle, although the nucleus can rarely be distinguished in fresh and unstained preparations, owing to the density of the blood pigment. The nucleus is usually single; infrequently there are two or three nuclei. In the case of *Glycimeris*, however, binucleated red cells are very common; tri- or tetranucleated ones are occasionally met with, and even penta- or hexanucleated ones are found with considerable frequency (Fig. 15, OHUYE, 1937 a). Small and anucleated red cells occur infrequently in the blood of *Travisia* (OHUYE, 1938 b). Whether these are the kind of erythroplastids described by ROMIEU (1923) or not is as yet uncertain. The adult red cells possess a small, round and finely granulated nucleus. But the young cells have a voluminous, round, oval or bean-shaped nucleus in which the chromatin granules assume an arrangement similar to a cartwheel (Figs. 6 & 11, OHUYE, 1937 a), such as is seen in the erythroblast

of vertebrates.

Cytoplasmic structure: The nature of the internal structure of mammalian erythrocytes is at present receiving considerable attention. From one point of view it is to be considered as a small jelly-like substance of homogeneous design, holding haemoglobin, etc. within the minute interstices of a relatively dense stroma (PRICE-JONES, 1920, etc.). From another point of view, the erythrocyte is to be considered as a balloon containing its elastic stroma and functioning substance as a relatively fluid colloid (PONDER, 1924, '25, etc.). The result obtained by this study of invertebrate erythrocytes seems to support the latter view. It is evident that the interior of the invertebrate erythrocyte is in a liquid phase, because the granular inclusions or crystals in the freshly withdrawn erythrocytes always an active Brownian movement (OHUYE, 1937 a). The fine internal structure was also demonstrated by the method of silver impregnation of TOMITA and others (OHUYE, 1937 a, b; 1938 b). Recently TOMITA and FUJITA (1937) stated as follows: " Die Erythrozyten sind bei den verschiedenen Tierarten überhaut kernhaltig, auch im Zellkörper mit einem feinmaschigen Netzwerk versehen, welches als Grundgerüst für die Erhaltung ihrer eigenen Form einer Rolle zu spielen vermag ". They found the reticulation pattern in the erythrocytes of fishes and amphibians, and assumed it as the fundamental type of fine structure from which other types may be derived in the course of ontogeny or phylogeny. That the reticulation pattern is almost constantly found in the erythrocytes of invertebrates would give, I think, a wider basis to the views of TOMITA and others on the evolution of erythrocytes. A pattern consisting of six spokes radiating from a center at geometrically exact intervals was found in the erythrocytes of *Siphonosoma* (Fig. 5, OHUYE, 1938 b). This so far as I am aware is regarded as a new type of which no previous description has been found. It is frequently considered that the fine structure of erythrocytes is no more than a mere artefact. But I think it is hardly possible to consider as an artefact such a regular and constant structure, as was found, for instance, in the erythrocytes of *Siphonosoma*. To explain the fact that the erythrocyte has a biconcave form, while a vesicle containing fluid would tend to assume a spherical form in the plasma, some inside structure should be considered; that is, there must be some structure preventing the erythrocyte from yielding to the stress of the surface forces acting upon it. From its fine and regular structure it seems to me that the pattern under discussion is well fitted to this purpose.

It is evident that the radiating pattern of *Siphonosoma* erythrocytes

is deformed due to some change of external condition.   It becomes irre-
gular in shape or disintegrates into fine particles by the action of partial
haemolysis (OHUYE, 1938 b).   In the majority of the invertebrates examined,
similar results were almost constantly observed (OHUYE, 1936 b, '37 a).
Such an inconstancy of pattern had already been pointed out by SHIBATA
(1933) and IZUMI (1935), working with erythrocytes of various vertebrates.
Accordingly I suppose that a pattern of this nature may be the reason
why the fine structure of erythrocytes is assumed by several authors to
be an artefact.

A definite membrane undoubtedly exists in the red cells of invertebrates.
It resists considerably the action of a hypotonic as well as of a hypertonic
solution, but it is relatively less resistant to that of saponin (OHUYE, 1937 a).
The cell membrane ruptures spontaneously within a few hours in a 0.003
per cent solution of saponin and the contents run out visibly through the
localized opening.

Staining: The red cells are constantly negative to the vital staining.
The supravital staining with neutral red causes the appearance of one to
several perinuclear granules which are similar to the so-called segregation
apparatus (OHUYE, 1936 b, '37 a, b, '38 b).   After prolonged exposure to
the dye, the induction granules which are fine and numerous appear
throughout the cytoplasm.   The mitochondria are almost constantly demon-
strated in the red cells with the supravital staining of Janus green B
(OHUYE, 1936 b, '37 a, b, '38 b).   Brilliant cresyl blue (OHUYE: Figs. 2, 5,
1936 a; Fig. 4, '37 b), Nile-blue sulphate, methylene blue, neutral violet
and cresyl red (OHUYE, 1937 a) have more or less affinity for the red cells.

The cytoplasm of adult red cells is stained, of course, an eosin pink
by the method of GIEMSA, while the young cells show polychromatic
reactions of various degrees (OHUYE, 1936 b, '37 a, b).

Blood pigments: The blood pigments of erythrocytes are distributed
uniformly throughout the cytoplasm.   Little is known of the factors in-
volved in maintaining the blood pigment within the red cell in solution.
Intracellular crystallization of the pigment was accidentally observed in
the red cells of *Urechis* (Fig. 3, OHUYE, 1937 a), *Travisia* (OHUYE,
1938 b), *Lingula* (Fig. 3, OHUYE, 1937 b) and *Arca* (Fig. 10, OHUYE,
1937 a).   The crystals are rhombic prism or rhombic tabular in shape,
and slightly yellow or yellowish pink in colour.

Granular inclusions: Granular inclusions were a constant element in
the red cells observed by me (OHUYE, 1936 b, '37 a, b, '38 b).   The cry-
stals (not of blood pigment) are seen in the red cells of *Physcosoma*

(Fig. 7, OHUYE 1937 a) and *Sipunculus* (OHUYE, 1938 b). Usually a single brown granule is found in those of *Caudina* and *Molpadia* (Figs. 1–4, OHUYE, 1936 b). These granules are relatively large, and show little or no Brownian movement. They are supravitally stained with neutral red, Nile-blue sulphate, indophenol blue, etc. (OHUYE, 1936 b, '37 a), but are negative to staining with Sudan dyes (OHUYE, 1936 b, '37 a). The red cells of *Urechis, Thalassema* and *Arca* also contain brown granules besides colourless ones (OHUYE, 1937 a). But they are fine and numerous, and essentially consist of haematin or a substance closely related to it. There is a tendency for these brown granules to increase with the age of animals (OHUYE, 1937 a). There are other animals that contain colourless or slightly yellowish granular inclusions, varying in number and size, and that exhibit active Brownian movements (OHUYE, 1936 b, '37 a, b, '38 b). Almost all granular inclusions show a positive reaction to fats (OHUYE, 1937 a, '38 b), oxidase (OHUYE, 1936 b, '37 a, b, '38 b) and vitamin C (Figs. 2 & 3, OHUYE, 1938 b). They are stained more or less with vital dyes. Vitamin A was demonstrated in those of *Urechis, Thalassema* (OHUYE, 1937 a) and *Travisia* (OHUYE, 1938 b). The results of tests for glutathione were always negative. That the granular inclusions contain such substances as lipoids, oxidase, vitamins A and C, etc., makes me conclude that they are not only accidental products of cell life, but are also an essential cell organ which probably plays a rôle in cellular respiration (OHUYE, 1938 b). Besides the granular inclusions, vitamin C granules are also demonstrable in the cytoplasm (OHUYE, 1938 b). They have an appearance similar to mitochondria, and show a remarkable reduction in number in the red cells of animals in prolonged captivity (Fig. 3, OHUYE, 1938 b).

Erythrocytopoiesis: I totally failed to detect the specialized erythrocytopoietic organ in any animal examined. The haemocytopoietic tissues of animals utilized in the present work were usually the epithelium lining the peritoneal cavity and digestive tract (Fig. 24, OHUYE, 1937 a). It was frequently seen that cells of the epithelium are phagocytic, argentophilic and intensely proliferating (OHUYE, 1937 a). The red cells may be essentially coelomic elements, as was pointed out by JORDAN (1933). Secondarilly these cells may migrate in part into the blood vascular channels.

Conclusion: That the granular inclusions are present almost constantly should be taken as a characteristic of invertebrate erythrocytes. These granular inclusions are similar to the granules of leucocytes in their chemical nature; that is, lipoids, vitamins A and C, and oxidase, which

may play an important rôle in the cellular respiration, are relatively abundantly and almost constantly found in these granular inclusions. Accordingly I suppose the erythrocytes of invertebrates are essentially modified leucocytes. This is also the view stated by JORDAN (1933). As a matter of fact, the coarsely granular leucocytes of *Ceratocephale* showed some transitional properties. Namely, they occasionally possess structures similar to the reticulation pattern and neutral red bodies of erythrocytes (OHUYE, 1938 a). The leucocytic characteristics, however, nearly disappeared in the erythrocytes of various invertebrates, and even these granular inclusions were hardly discernible in the erythrocytes of *Siphonosoma* (OHUYE, 1938 b), *Lingula* (OHUYE, 1937 b), etc. Furthermore genuine erythroplastids comparable to the erythrocytes of mammals are also described by several authors. Elastic membrane, changeable osmotically and chemically, also exists in invertebrate erythrocytes. Mitochondria, reticulation patterns, neutral red bodies and so forth are demonstrable in these cells too. All things considered, it seems that no real gap exists between erythrocytes of invertebrates and those of vertebrates. Accordingly it is incorrect to say that among the invertebrates there is no cell which is exactly comparable with the erythrocytes of vertebrates, as is frequently stated in text-books.

B. LEUCOCYTES

a) Hyaline Leucocytes or ' Lymphocytes '

These are an almost constant element in the blood or coelomic fluid of invertebrates, and possess nearly transparent, colourless and homogeneous cytoplasm. Two main types of cells are distinguishable: The first type is characterized by a scanty cytoplasm and a relatively large nucleus ·· cells of the lymphocyte type. The second type has an abundant cytoplasm and an excentric, oval, kidney- or horseshoe-shaped nucleus - - cells of the monocyte type. Such classification is, of course, not absolute, and there are a good many intermediate forms between these types of cells, as will be seen later.

Among the cells of the lymphocyte-type, three subtypes of different sizes are distinguished — the small, the medium-sized and the large lymphocytes. These groups cannot be so distinctly separated from each other. The small lymphocytes are of the most common variety. The average diameter of these cells is approximately 10 μ; that of the large lymphocytes may reach 14 μ or more. The medium-sized ones are, of course,

the intermediate size between these two cells. In the vertebrates, it is well known that the large lymphocytes are normally found in the blood forming organs, but not in the blood itself. In the invertebrates, however, they occur frequently in the circulating blood (OHUYE: Fig. 2, 1936 a, Fig. 6, '36 b). The difference between the large lymphocytes and the monocytes is also indistinct in the invertebrates. I presumably classified ' monocyte ' in my previous papers (OHUYE, 1934 b, '36 a, b, '37 a) as a cell which is large in size and possesses a nucleus which is relatively less voluminous and excentric, as was mentioned just above. It is generally accepted in the vertebrate haematology that the lymphocytes are constantly negative to the Nadi-reaction while the monocytes are more or less positive to this reaction. No such difference is discernible in the invertebrates. Accordingly, to classify the hyaline leucocytes of the invertebrates in the manner mentioned above is not so essential as in the case of the vertebrates. In the invertebrates, it is also difficult to distinguish to monocyte from the histiocyte, because the cells are very similar to each other in size, structure and function. Accordingly I have given no information concerning the histiocyte. The nucleus of the small and medium-sized lymphocytes contains large, irregular and darkly staining lumps of basichromatin with narrow spaces between them (Figs. 1–4, Pl. II, OHUYE, 1934 b). The chromatin particles are frequently arranged in the form of the spokes of a wheel (Fig. 18, OHUYE, 1937 a), showing the relation of these cells to the erythroblasts. A few azurophilic granules are occasionally met with in the cytoplasm of small lymphocytes (Fig. 2–3, Pl. IV, OHUYE, 1936 d). In the large lymphocytes the cytoplasm forms a wider zone around the oval or kidney-shaped nucleus. As a rule, this nucleus stains by GIEMSA's method much lighter than that in the small lymphocytes. These findings on the invertebrate lymphocytes are in accordance with those of the vertebrate lymphocytes.

The chromatin granules of monocytes are usually finer and more numerous than those in the lymphocytes. But occasionally they are coarse and not numerous (OHUYE, 1934 b, '36 a). One or two small nucleoli are frequently present, although not seen in dry smears (OHUYE, 1936 a).

The lymphocytes, including monocytes, are positive to vital staining with carmine, trypan blue and so forth (OHUYE, 1934 a, b, '36 a–d, '37 a). The mitochondria are usually accumulated on one side of the nucleus (Fig. 18, k–m, OHUYE, 1937 a). Vacuoles stainable supravitally with neutral red, brilliant cresyl blue, etc. are also present (OHUYE: Fig. 2, 1934 a; Fig. 1, '36 c; Fig. 8, '37 a). The very abundant existence of

vitamin C granules is found in these cells (OHUYE, 1938 a). They are
very similar to the mitochondria in appearance and arrangement. Need-
less to say, the lymphocytes are stained basophilically by the method of
GIEMSA.   The lymphocytes are capable of active amoeboid movement,
protruding lobular, membranous, rod-like, or needle-shaped pseudopodia.
The last two are recognized as abnormal (OHUYE, 1934 a, b). The ability
of phagocytosis is also most active in these cells (OHUYE, 1934 a). The
lymphocytes form a coagulum together with other formed elements when
the body fluid has been removed from the animal body and allowed to
stand a few minutes in a watch glass (OHUYE: Fig. 1, 1934 a ; Fig. 6.
'36 b ; Fig. 2, '36 c). Real syncytium formation was found in the blood
cells of Crustacea, but in most of the cases of other animals, the bound-
aries of cells forming the coagulum evidently remain.

It seems to me that the lymphocyte of invertebrates is a pluripotential
cell from which at least some granulocytes, crystal cells, vesicular cells,
compartmental cells, etc. might be produced. This will be discussed a
little later.

In short, the lymphocytes of invertebrates are active in various func-
tions, and still remain in the primitive stage in which lymphatic and
myeloid differentiation does not occur.

b) Granulocytes

The leucocytes with colourless or slightly yellowish specific granules
are included in this group. These are also one of the formed elements
which almost constantly appear in the body fluid of invertebrates. The
following kinds of cells described in my previous papers (OHUYE, 1934 a, b ;
'36 a–d ; '37 a–c ; '38 a) are included in this group ; amoebocytes with
colourless granules (*Caudina*), morula cells (ascidians), granular amoeboid
corpuscles (ascidians, *Molpadia*) granular amoebocytes (animals described
in OHUYE, 1936 c, '37 a), granulocytes (animals described in OHUYE, 1934 b,
'37 a, b, '38 a), and lamprocytes (earthworms). In a previous paper (OHUYE,
1938 a) I classified the granulocytes as follows : A. granulocytes with fine
granules, B. granulocytes with coarse granules, C. granulocytes with both
kinds of granules, and D. pigmented granulocytes. In this paper, however,
the last one is described independently in an other section.

Size : The size of the granulocytes shows a great deal of variation
such as 6 8 μ in diameter (small granulocytes of *Physcosoma*, Fig. 19,
OHUYE, 1937 a) to 30 μ and more across (coarsely granular leucocytes of

Ceratocephale, Fig. 2, OHUYE, 1938 a). The majority of granulocytes, however, are 10–16 μ in diameter.

Nucleus: The granulocytes usually possess a single, round or oval nucleus which is frequently obscured from sight, owing to the existence of numerous specific granules. The nucleus is usually small, but occasionally it amounts to the largest part of the cell volume (granular leucocytes of some Crustacea; for instance, those of *Spirontocaris*, Fig. 9, OHUYE, 1938 a). Since the chromatin net-work is woven loosely, the nucleus stains lighter in shade with basophilic dyes than the nucleus of lymphocytes does (OHUYE, 1934 b, '36 d). Occasional granulocytes of Cephalopoda possess deeply indented, horseshoe-shaped, or bi- or trilobular nuclei (Fig. 7, OHUYE, 1938 a). I have discussed these so-called polymorphic nuclei in a previous paper (OHUYE, 1938 a).

Cytoplasm: In the freely moving granulocyte the cytoplasm is seen to consist of a hyaline exoplasm which forms the advancing pseudopodia and into which there flows an entoplasm containing numerous specific granules. Aside from these granules in the entoplasm we may frequently find one or more vacuoles of varying sizes (OHUYE: Fig. 17, 1937 a; Fig. 8, '37 b), which are stainable supravitally with dyes. Vitamin C granules are also demonstrable in the entoplasm. They are round spherules or rods, varying in number and size, and are either scattered throughout the entoplasm or show more or less of a tendency towards perinuclear condensation (Figs. 1, 2 & 9, OHUYE, 1938 a). Oxidase granules in the cytoplasm may be detected by the Nadi-mixture (OHUYE, 1936 a–d; '37 a–c; '38 a). A few oil droplets are occasionally met with in the cytoplasm (OHUYE, 1937 a).

Granules: Specific granules varying in size, shape and number are found in the entoplasm. They are usually round or oval, but rod- or bacillus-like granules are encountered infrequently (*Drawida*, Fig. 15, Pl. II, OHUYE, 1934 b; *Euphrosyne*, Fig. 2, OHUYE, 1938 a; *Physcosoma*, Fig. 19, 1937 a, Fig. 3, '38 a, OHUYE; *Idiosepius*, Fig. 2, OHUYE, 1938 a). The smallest is less than 1 μ in diameter (small granulocytes of *Physcosoma*) while the largest exceeds 3 μ and more across (coarse granular cells of *Ceratocephale*). The terms coarsely granular leucocytes and finely granular leucocytes were used for the granulocytes whose granules are uniformly large or small. The morula cell of ascidians should be recognized as a coarsely granular leucocyte. Occasional granulocytes of echinoderms, gephyreans, etc. possess one or several granules of brown, yellow, green or some other colours, besides the colourless specific granules. These cells are also classified into either of the two granulocytes, according to

the size of the specific granules. Simultaneous occurrence of fine and coarse granules in a cell is occasionally encountered (*Tachypleus*, OHUYE, 1938 a; *Pheretima*, OHUYE, 1937 c). Some granulocytes contain only a few granules (coarsely granular cells of *Urechis*, *Thalassema*, OHUYE, 1937 a; *Libitina* and *Ceratocephale*, OHUYE, 1938 a) while the usual granulocytes are so densely packed with granules that the nucleus is entirely concealed by them. The refringency of granule shows also a considerable variation in different cells. The highest refractive granules are, perhaps, seen in the lamprocytes of earthworms (OHUYE, 1934 b, '37 c), while the lowest is visible in the leucocytes of *Temnopleurus* (OHUYE, 1936 c), although I did not made any exact estimation of the refringency of the granules. Such differences may suggest the diversity of the chemical constituents of the granules. In fact the granules in the lamprocyte show an intense reaction to fats while those in the coarse granulocytes of *Temnopleurus* show a positive reaction to MILLON's test (OHUYE, 1936 c). Trials to stain the granules of leucocytes with Sudan dyes have been made by many workers, but they have usually ended in failure in the experiments using invertebrate granulocytes. In the present investigation, however, the staining of the granules following the method of KAWAMURA and YASAKI (1933) succeeded almost constantly (OHUYE, 1938 a). The granules are also positive to supravital staining with indophenol blue (OHUYE, 1938 a). The Nadi-mixture stains them almost constantly (OHUYE, 1936 a–d; '37 a–c; '38 a). The blackening of granules by the action of osmic acid is encountered rather infrequently. From the solubility in various fat-solvents, it is supposed that some granules are composed essentially of glycerin ester, cholesterin ester, etc. (coarse granules in leucocytes of *Tachypleus*, for instance), while the other may be formed chiefly of phosphatids (fine granules of leucocytes in the same animal). The detection of vitamin A in the granules succeeded in the granulocytes of some gephyreans (OHUYE, 1937 a), of some star fishes and of *Tachypleus* (OHUYE, 1938 a), upon very rare occasions. With a few exceptions, the vitamin C reaction of granules is usually negative, while this substance is easily demonstrable in the intergranular cytoplasm of all granulocytes (OHUYE, 1938 a). Granules stained with iodine vapour and BEST's carmine are encountered in the granulocytes of *Tachypleus* (OHUYE, 1938 a). The reaction of glutathione ended in failure.

Genuine basophilic granulocytes are found in the body fluid of *Drawida*, *Molpadia*, *Caudina*, *Terebratalia*, *Coptothyris*, *Ostrea*, *Anomia*, *Mytilus*, etc., and genuine eosinophilic granulocytes are met with in that of *Drawida*,

Pheretima, Euphrosyne, Lingula, Terebratalia, etc. That the eosinophilic granulocytes are very abundantly found in the blood of annelids is interesting (OHUYE, 1934 b, '37 a, c). In occasional granulocytes of Cephalopoda, are embedded both eosinophilic and basophilic granules in various proportions (OHUYE, 1938 a). The great part of invertebrate granulocytes, however, show an amphophilic reaction with more or less inclination towards either basophilic or eosinophilic (OHUYE, 1937 a, '38 a), as has been stated by many authors. This fact suggests that the granulocytes of invertebrates remain in a lower degree of differentiation, as compared with those of higher vertebrates in which the amphophilic granules are seen only in the embryonic stage.

Motility and other characters: The granulocytes are amoeboid, as was mentioned above. Shape of pseudopodia shows a remarkable variation, as was the case of lymphocytes. Occasional cells show a great elongation of the cell body in the case of movement, the length of the elongated cell reaching 180 μ and more in an extreme case (finely granular cells of *Styela,* Fig. 7, OHUYE, 1936 a). The amoeboid movement is more active in the finely granular leucocytes than in the coarsely granular ones (OHUYE, 1938 a). Such difference is very distinct with respect to phagocytosis; namely the former remarkably ingests India ink in vitro while the latter usually takes almost nothing (OHUYE, 1938 a). The granulocytes also form coagulum on the outside of the animal body, but no real syncytium is discernible except in the case of Crustacea (OHUYE, 1934 a, '36 c, '38 a).

Conclusion: It is evident from the above statement that the properties of invertebrate granulocytes show a remarkable diversity as against those in vertebrates, and the granules have a varied size, shape and staining reaction. Some authors (KIYONO, 1929, for instance) have not believed in the existence of genuine boso- or eosinophilic granules in the invertebrates. These granules, however, are found in the present investigation. As was pointed out by KIYONO (1929), the abundant existence of amphophilic granulocytes is a characteristic of invertebrate blood. As the amphophilic granules show a tendency towards either baso- or eosinophilic (OHUYE, 1938 a), it is supposed that they still remain in the course of differentiation to the monochromatophilic granules. The polymorphic nucleus, which is, needless to say, very common in the vertebrate granulocytes, is encountered very infrequently in invertebrates. This fact may also be taken as evidence indicating the low differentation of invertebrate granulocytes. The granular leucocytes abundantly contain various sub-

stances which may play an important rôle in regard to the oxido-reduction in the cells. This, I think, would serve, more or less, to interpret the function of granulocytes.

c) Pigment Leucocytes

In this group, leucocytes with distinctly coloured and constantly existing granules are involved. The following animals possess cells belonging to this group; the holothurids, star fishes (brown amoebocytes, OHUYE, 1934 a; '36 b, c; '38 a), a polychaete, *Potamilla* (green cells, OHUYE, 1938 a), the brachiopods (red, orange and brown amoebocytes, OHUYE, 1936 d, '37 b, '38 a), a nudibranch, *Cuthona* (green cells, OHUYE, 1938 a), the ascidians (green, brown, orange and grayish olive cells; OHUYE, 1936 a). Pigmented leucocytes are in general similar to the coarse granular cells in size and appearance. The nature and function of these cells, however, show a good deal of variation. The pigment of brown amoebocytes of echinoderms is probably a lipochrome, judging from its reaction to acids, alkali, fat-solvents, Sudan dyes, indophenol blue, osmic acid, etc. (OHUYE, 1936 c, '38 a). The brown granules show an intense reaction of vitamin C (OHUYE, 1938 a). The green granules in the leucocytes of *Potamilla* and *Cuthona* exhibit similar reactions to various reagents (OHUYE, 1938 a). They are stainable with Sudan dyes when the cells have been previously immersed in alkali, but hardly soluble in fat-solvents. Various acids cause the green granules to dissolve or to discolour. Vitamin C reaction is positive in almost all granules of green cells of both animals (OHUYE, 1938 a). That the brown and green coloured granules contain abundant vitamin C contrasts with the aforementioned colourless granules in which this substance was usually undemonstrable. By an optic section of green granules it is seen that the pigment is localized superficially (OHUYE, 1938 a). The orange granules found in the leucocytes of brachiopods may also contain a lipochrome, as they are blackened by osmic acid (OHUYE, 1936 d). The red granules in those of brachiopods contain presumably an echinochrome, judging from the colour, solubility, and characteristic reactions to alcohol and to caustic soda (OHUYE, 1936 d). The reaction of vitamin C is also positive in these cells (OHUYE, 1938 a). Numerous yellowish gray-coloured granules are embedded in the cell body of brown granular amoebocytes of brachiopods (Figs. 26–29, Pl. IV, OHUYE, 1936 d). The pigment of these granules is probably a lipochrome too. These brown granules give the most intensive reaction to vitamin C among all the granules of pigmented leucocytes (OHUYE, 1938 a). It is noteworthy that the reaction

of glutathione also occurs very intensely in these granules (OHUYE, 1938 a). The brown amoebocytes are found in the subepitherial tissue of cirri (Fig. 2, OHUYE, 1937 b), standing in juxta-position to the red granular cells found in the axial blood vessels of the cirrus. The facts that the brown granules contain various respiratory substances such as lipoids, oxidase, vitamin C, glutathione, etc., that the red granules contain echinochrome (?), vitamin C, lipoids, oxidase, etc., and that both these cells are localized in the cirri in what may be taken as a respiratory organ, make me suppose the existence of an important correlation between these cells and the respiratory function of animals. That is, the brown amoebocytes may act, I believe, to deliver oxygen in the respiratory medium to the red cells in blood vessels through the wall of the cirrus.

As to vitamin C and glutathione, some very interesting investigations are being conducted by KLIGLER and his co-worker. KLIGLER (1936) has shown that the addition of a very small amount of ascorbic acid to cultures of *C. diphtheriae* leads to the production of relatively atoxic filtrates. Recently KLIGLER and BERNKOPF (1937) have ascertained that ascorbic acid possesses also the inactivating action of vaccina virus. Similar tests were made by these authors (1937) with glutathione also. This substance, however, proved much less active than ascorbic acid. Such inactivation is supposed to be owing to the fact that the chemical composition of viruses and diphtheria toxin may be effected by those oxido-reducing substances. With respect to this fact the abundant existence of vitamin C and glutathione in the brown amoebocytes is full of interest. And it is a cause for consideration that the significance of extensive distribution of vitamin C, which is most active regarding oxido-reduction, throughout the red and white blood corpuscles of invertebrates should be sought from this point of view.

It is a generally accepted fact that various pigments found in ascidian leucocytes are derivatives of vanadium compounds. In the present work (OHUYE, 1936 a) the pigments show vanadium reactions too.

Almost all pigmented leucocytes are more or less amoeboid and phagocytic, and positive to the Nadi-reaction and to supravital staining with various dyes (OHUYE, 1934 a, b; '36 a d; '37 a-c; '38 a).

d) Specially Formed Elements

Cells remarkably deviated from ordinary blood corpuscles are found in the body fluid of some invertebrates, and the following cells may be classified in this group; vesicular amoeboid cells (ascidians, Fig. 6, OHUYE,

1936 a ; echinoderms, Fig. 9, 1936 b ; Fig. 5, 1936 c, OHUYE), compart-
mental cells (ascidians, Fig. 5, OHUYE, 1936 a ; holothurids, Fig. 6, OHUYE,
1936 c), crystal corpuscles (holothurids, Fig. 10, OHUYE, 1936 b), spindle
bodies or fusiform corpuscles (holothurids, Fig. 12, OHUYE, 1936 b ; brachio-
pods, Figs. 1, 8 & 9, OHUYE, 1937 b), linocytes (earthworms, OHUYE :
Figs. 16 & 17, Pl. II, 1934 b ; Fig. 2, 1937 c) and urns or ciliated cor-
puscles (sipunculids, Fig. 20, OHUYE, 1937 a). The three kinds of cells
first mentioned, I suppose, are derived from the cell of the lymphocyte-
type. The origin, function, fate, etc., of spindle bodies are yet uncertain.
The linocyte is found only in the body fluid of the earthworm. From
the staining reaction and characteristic myelin forms in the cytoplasm,
the lineal structure of the cell is supposed to consist essentially of lipoids
(OHUYE, 1937 c) but its function is as yet quite unknown. As to the
urn, I have no newly discovered facts about it except that this enigmatic
body contains numerous eosino-, osmo-, sudanophilic, and oxidase-positive
granules (OHUYE, 1937 a, '38 a), and that it is the only cell negative to
vitamin C reaction (OHUYE, 1938 a) in all the blood cells examined in
the present work.

Short remarks on the invertebrate leucocytes. Taking a general survey
of invertebrate leucocytes, the following facts are noticeable : 1. Various
pigmented cells are abundantly found in the body fluid of various animals.
2. The varieties of leucocytes are remarkably numerous. 3. The ampho-
philic granulocytes occur extensively in the blood of adult animals.
4. The polymorphic nucleus is encountered upon very rare occasions.
5. The Nadi-reaction is positive in almost all leucocytes. The last three
characteristics mentioned may indicate the low differentiation of the leuco-
cytes. KIYONO (1929) also stated similar findings concerning the haemo-
lymph cells of invertebrates. As to the pigmented leucocytes, so far as
I am aware, only white corpuscles with lipofuscin are indicated in the
normal vertebrate blood. In the invertebrates, however, the various pig-
mented leucocytes are rather common constituents, and it is easy to find
animals whose blood contains three or more kinds of these cells simul-
taneously (Brachiopoda, Tunicata, etc.). That there exist numerous varieties
of leucocytes in the invertebrates is due, perhaps, to the existence of the
more varied modes of life and different organizations of these animals as
compared with the vertebrates. That there remain many primitive types
of cells in invertebrates which would have disappeared in vertebrate blood
may be supposed as a further cause of the phenomenon of different kinds

of cells. For the basis of such supposition, the following fact is indicated: The red granular and brown granular leucocytes which play an important rôle in respiration are constantly found in two brachiopods having no erythrocytes (*Terebratalia* and *Coptothyris*), while no such cell is visible in the body of another brachiopod possessing erythrocytes as respiratory cells (*Lingula*).

Whether the granules of colourless or pigmented leucocytes are the product of mitochondrial differentiation or not is an interesting but at present a still unsolved question. SABIN and her co-workers (1924) believed that, in the transformation of the myelocyte into the granulocyte, there is a general reduction in the number of mitochondria as the specific granules increase in number (qutoted from COWDRY, 1932). Recently the chemical properties of mitochondria were studied by JOYET-LAVERGNE (1935), BOURNE (1935), etc., and it was made clear that mitochondria contain vitamin A, vitamin C and glutathione, besides other well known substances such as proteins, lipoids, enzyme of MARSTON, etc. In the present investigation, the majority of specific granules of colourless granulocytes showed no reaction to vitamin C (OHUYE, 1938 a). Accordingly it may be supposed that they are not composed of the substance produced by a direct metamorphosis of mitochondria. In regard to the brown granulocytes of brachiopods, which show the most intense reaction of vitamin C and gluathione, there is a probability of this transformation. The blood cells of brachiopods are, therefore, very interesting on this point.

Most of the various kinds of formed elements are found in the body fluid of earthworms. The blood and coelomic corpuscles of earthworms hitherto reported by many authors are as follows; lymphocytes, monocytes, eosinophiles, basophiles, amphophiles, spindle bodies, lamprocytes, eleocytes, linocytes, mucocytes, chloragocytes, detached peritoneal cells, etc. The five kinds of cells mentioned first are the most common elements found in the blood of both invertebrates and vertebrates. The rest of the cells may be coelomic elements and most of them are specific to earthworms. They would have disappeared in higher animals in the course of evolution. In fact, Polychaeta and Sipunculoidea, which bear a close relation to earthworms, have blood corpuscles similar to those of vertebrates, except for the presence of urns in the latter. The Polychaeta possesses haemoglobin as the respiratory pigment, while the Sipunculoidea contain haemerythrin, so that the course of evolution would have taken the direction from Oligochaeta to Polychaeta. As was pointed out in my last paper (OHUYE,

1938 b), the following successive stages of blood evolution will be found in Polychaeta ; a) haemoglobin is dissolved in plasma (*Nereis*, for example), b) haemoglobin is contained in both plasma and erythrocytes (*Travisia*), c) haemoglobin is found only in the erythrocytes (*Glycera*), and d) non-nucleated erythrocytes or erythroplastids are present (a polychaete, *Magelona*, ROMIEU, 1923). Thus the annelids seem to occupy an important situation with respect to the blood evolution also.

In the present investigation, it is interesting to note that the animals having erythrocytes are all those which live by being embedded in sand or mud. The evolution of erythrocytes may, probably, have some relation to such a mode of life.

The echinoderms and annelids possess relatively well differentiated leucocytes, irrespective of either the presence or the absence of erythrocytes. However there is noted a general tendency that the animals with erythrocytes possess less differentiated or less varied leucocytes than those animals having no erythrocytes. The brachiopods mentioned just above are an appropriate instance showing this relation. In the molluscs, a similar relation is discernible between the *Arca* and the *Ostrea* (OHUYE, 1937 a, '38 a). The blood of crustaceans is alike to that of lamellibranchs and cephalopods in the points that granulocytes are found abundantly, and that pigmented leucocytes and specially formed elements are usually invisible (OHUYE, ·1938 a). An abundant existence of pigmented leucocytes in the ascidian blood reminds us of the blood of brachiopods. As was stated in previous papers (OHUYE, 1936 a, d ; '37 b), the pigmented leucocytes of these animals may serve as respiratory cells. Such an analogy of blood is probably due to the sedentary life of these animals.

The lymphocyte occurs in almost all metazoa --- from the sponge (JORDAN, 1933) to the amphioxus (SHIMA, 1933), and shows a very low differentiation from ordinary cells. I suppose this is the most primitive and generalized cell in the body fluid of metazoa, and may serve as the stem-cell in the blood-cell evolution. Granulocytes are also a nearly constant element of body fluid throughout invertebrates except in the very lowest (CUÉNOT, 1891, JORDAN, 1933, KOLLMANN, 1908, KIYONO, 1929, OHUYE, 1938 a, etc.). It is supposed from their structure and function that the granulocytes may derive from the lymphocytes. In comparison with the functions of both cells, the amoeboid movement, the phagocytosis, the thrombogenesis, etc. are less active in the granulocytes than in the lymphocytes. Such a decrease of functions is generally proportional to the increase of the number of specific granules in the cytoplasm of granulo-

cytes. KINDRED (1926), working with *Arbacia*, has already stated the relation of both cells as follows: "Amoebocytes with colourless spherules (= granulocytes) are inferred to arise from leucocytes (= lymphocytes) which have ingested food." As to the structure, various transitional stages between granulocytes and lymphocytes are visible (OHUYE, 1931 a, '37 a, '38 a). The relation between granulocytes and erythrocytes was mentioned above. That the eosinophilic granulocyte may possess some relation to the erythrocyte is inferred from the following: OKAJIMA's method (OKA-JIMA, 1916) believed to be specific for haemoglobin staining is also positive to eosinophilic granules (ROMEIS, 1928; KINDRED, 1932; OHUYE, 1936); KINDRED (1932) pointed out the existence of the parallelism of the staining reaction with various dyes (eosin Y, light green, Bordeaux red, aniline blue, acid fuchsin and orange red) among red blood corpuscles, heme smears, and acidophilic granules. The close relation between lymphocytes and erythrocytes is surmised from the evidence that the erythroblast possesses properties very similar in the chromatic reaction, nuclear struc-ture, etc, to the lymphocytes (OHUYE, 1937 a). Pigmented leucocytes are, perphaps, lymphocytes or granulocytes with coloured granules. Most of the specially formed elements of the body fluid may be also modified lymphocytes (vesicular cells, compartmental cells, crystal corpuscles, etc.) or detached peritoneal elements (urns). As to the spindle bodies, whether these cells originate from muscular tissue or from red blood cells is as yet uncertain. I have discussed this question in a previous paper (OHUYE, 1937 b). Referring to the facts mentioned just above, I presumed to make a scheme which may show probable phylogenetic relations among the formed elements in the body fluid of invertebrates.
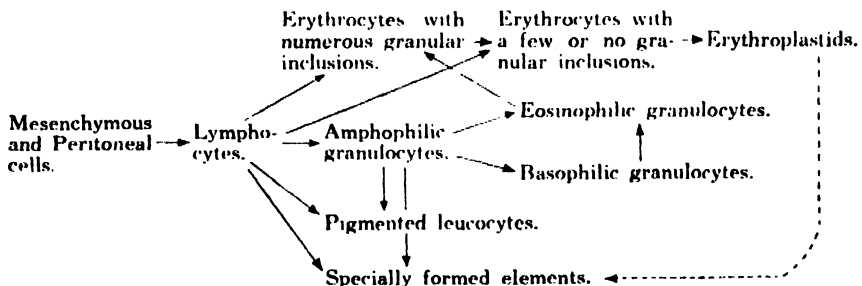
A survey of literature on this subject suggests the conclusion that the lymphocyte of vertebrates is a pluripotential element capable of varied differentiations dependent severally upon the specific stimuli of a varied environment. The lymphocyte may become a macrophage (MAXIMOW,

1916 - quoted from JORDAN, 1923); it may differentiate into a fibroblast (CARNEL and EBELING, 1922); or it may provide for the replenishment of the various types of blood corpuscles (JORDAN, 1923, '24 a, b, '25, '26). Thus even the lymphocyte found in highly differentiated vertebrates serves as the mother-cell of various formed elements. Therefore, it seems to be reasonable to conclude that the lymphocytes is the stem-cell of blood-cell evolution in the invertebrates in which the haemocytopoiesis still remains in a primitive condition.

As is seen in the above statement, the variety of cellular elements in the blood of invertebrates is rather abundant as against that of vertebrates. However, the blood cells of these animals remain in a more or less primitive stage, and many intermediate types concerning cellular structure and function are usually found in each kind of cells. In other words, the transformation of one type of cell into another type may be possible, within a certain limit, in the body fluid of adult and healthy invertebrates. This is in accordance with KIYONO's opinion (KIYONO, 1929) that the haemolymph cells of invertebrates are similar to the embryonic blood cells of vertebrates in many points. It is regarded as a matter of course that the invertebrates possess such less differentiated blood cells in comparison with the vertebrates.

SUMMARY

General investigations were made of the formed elements in the body fluid of 44 invertebrate animals which cover six phyla; Echinoderma, Annelida, Molluscoidea, Mollusca, Arthropoda and Prochordata. There is no fundamental hiatus between the erythrocytes of vertebrates and those of invertebrates. Granular inclusions are constantly found in invertebrate erythrocytes. They are considered to be an analogous substance to the granules of invertebrate leucocytes from the point of their chemical properties. Accordingly it is supposed that the erythrocytes are essentially modified leucocytes. The lymphocyte may be a pluripotential element capable of a varied differentiation dependent severally upon the environmental factors, and it is supposed that this cell may be the progenitor of other formed elements in the invertebrate body-fluid. Granular leucocytes are very similar to those of higher vertebrates except that the polymorphic nucleus is difficult to find, and that the amphophilic granulocytes are found extensively in the adult animals. The existence of abundant and various pigmented leucocytes and specially formed elements may be taken as a characteristic of invertebrate blood.

ACKNOWLEDGMENT

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SYMBOLAE ITEOLOGICAE VI

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(Cum 3 tabulis et 2 figuris in textu)

(Opus acceptum d. XV m. Novembris a. MCMXXXVIII)

52) **Salix Warburgii** SEEMEN in ENGLER, Bot. Jahrb. XXIII. Beibl. LVII. p. 43 (1897).-- BURKILL in Jour. Linn. Soc. XXVI. p. 534 (1899) (FORBES & HEMSLEY, Ind. Fl. Sin. II.). --MATSUMURA & HAYATA in Jour. Coll. Sci. Tokyo, XXII. p. 395 (1906) (Enum. Pl. Formos.).---? LÉVEILLÉ in Bull. Soc. Bot. France LVI. p. 301 (1909).- KAWAKAMI, List Pl. Formos. p. 111 (1910). --MATSUMURA, Ind. Pl. Jap. II. 2, p. 16 (1912).—HAYATA, Gen. Ind. Fl. Formos. p. 73 (1917). - KANEHIRA, Formos. Trees, ed. 1, p. 592 (1918); ed. 2, p. 78 (1936).-- MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1131 (1925); ed. 2, p. 174 (1931). - KANEHIRA & SASAKI in Jour. Soc. Trop. Agr. IV. 3, p. 318 (1932) ut *Salix Warburgi*.

Syn. *Salix Oldhamiana* HENRY[1] (non MIQUEL) in Trans. As. Soc. Jap. XXIV. suppl. p. 90 (1896) quoad HENRY n. 1403, n. 1473.- -SCHNEIDER in SARGENT, Pl. Wilson. III. p. 100 (1916) pro syn. *S. Kusanoi*. **Syn. nov.**

Salix Mesnyi BURKILL[2] (pro parte non HANCE) in Jour. Linn. Soc. XXVI. p. 530 1899; FORBES & HEMSLEY, Ind. Fl. Sin. II.) quoad pl. ex Formosa.-- HAYATA in Jour. Coll. Sci. Tokyo, XXX. art. 1, p. 306 (1911) (Mat. Fl. Formos.); Gen. Ind. Fl. Formos. p. 73 (1917).- KUDO & MASAMUNE in Ann. Rep. Taihoku Bot. Gard. II. p. 6 (1932) (Gen Pl. Formos. I.).

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Salix glandulosa var. *Warburgii* (SEEMEN) KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 88 (1913). --SCHNEIDER in SARGENT, Pl. Wilson. III. p. 99 (1916).- -MATSUMURA, Shokubutsu-Mei-I, ed. 9, II. p. 352 (1916). -SASAKI, List Pl. Formos. p. 142 (1928).--- KUDO & MASA-

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[1] Vidi specimina HENRYANA n. 1404 et n. 1473 per benevolentiam Prof. A. REHDER, cui hoc loco permultas gratias ago.

[2] Specimen OLDHAMIANUM n. 509 ad *S. Warburgii* SEEMEN pertinere dicitur (sec. SCHNEIDER). Suum n. 510 mihi nondum visum. Japonicorum auctorum "*Salix Mesnyi* HANCE" est certe *Salix Warburgii* SEEMEN.

MUNE in Ann. Rep. Taihoku Bot. Gard. II. p. 6 (1932) (Gen. Pl. Formos. I.).— SUZUKI in MASAMUNE, Short Fl. Formos. p. 39 (1936). NEMOTO, Fl. Jap. Suppl. p. 107 (1936).

Salix Kusanoi SCHNEIDER in SARGENT, Pl. Wilson. III. p. 100 (1916) quoad specim. HENRY n. 1404, 1473. Syn. nov.

NOM. JAP. *Taiwan-yanagi* KAWAKAMI, List Pl. Formos. p. 111 (1910). *Takasago-Akame-yanagi* KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 88 (1913).—*Hansu-yanagi* HAYATA, Gen. Ind. Fl. Formos. p. 73 (1917).

HAB. in Japonia. *Formosa.*— Prov. **Taihoku**: Taihoku, (U. FAURIE n. 519 fr. 30 Apr. 1903 in Herb. Univ. Imp. Kyotensis; n. 1656 fr. Feb. 1915 in Herb. Univ. Imp. Tokyo.— T. NAGASAWA n. 457 ⚥ & ♀ 17 Jan. 1905 in Herb. Univ. Imp. Kyotensis. K. ODASHIMA ⚥ & ♀ 27 Jan. 1937. G. MASAMUNE ⚥ 20 Jan. 1937; ⚥ 27 Jan. 1938; st. 15 Mart. 1938.— T. HOSOKAWA n. A 316 fr. 25 Mart. 1938); Sitisei, (G. MASAMUNE n. 485 ⚥ & n. 486 ♀ 4 Jan. 1930); Sirin, (B. HAYATA fr. 30 Mart. 1916 in Herb. Univ. Imp. Tokyo.); Tamsui, (A. HENRY n. 1473 st. & n. 1404 ⚥ fl. in Herb. Arb. Arnold.— G. MASAMUNE st. 15 Apr. 1938); Urai, (B. HAYATA fr. & st. "Leaves glaucous beneath, dark-green slightly shining above" 4 Maio 1916 in Herb. Univ. Imp. Tokyo.— Y. HORIKAWA st. 12 Aug. 1932). Prov. **Sintiku**: Sintiku, (S. KITAMURA ⚥ 2 Feb. 1932); Tćen, (G. MASAMUNE n. 363 st. 9 Dec. 1929); Korisho, (U. FAURIE n. 21 fr. Mart. 1914 in Herb. Univ. Imp. Tokyo).

53) **Salix Kusanoi** (HAYATA) SCHNEIDER in SARGENT, Pl. Wilson. III. p. 100 (Maio 1916) pro parte: excl. synonym. *Salice Oldhamiana* HENRYI, *Salice tetrasperma* BURKILLI et descriptione SCHNEIDERI speciminibusque HENRYANIS. MAKINO & NEMOTO, Fl. Jap. ed. 2, p. 167 (1931) pro parte. — KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. X. p. 565 (1935) (Symbol. Iteolog. I.) pro parte: excl. synonym. *Salice Oldhamiana* HENRYI et *Salice tetrasperma* BURKILLI ceterorumque auctorum.

Syn. *Salix tetrasperma*, var. *Kusanoi* HAYATA in Jour. Coll. Sci. Tokyo, XXX. art. 1, p. 305 (1911) (Mat. Fl. Formos...

Salix suishaensis HAYATA, Icon. Pl. Formos. VI. p. 65 (Dec. 1916); Gen. Ind. Fl. Formos. p. 73 (1917).— MAKINO & NEMOTO, Fl. Jap. ed. 1. p. 1130 (1925); ed. 2, p. 172 (1931).— SASAKI, List Pl. Formos. p. 143 (1928). KUDO & SASAKI in Ann. Rep. Taihoku Bot. Gard. I. p. 23 (1931).— KANEHIRA & SASAKI in Jour. Soc. Trop. Agr. IV. 3, p. 317 (1932).— KUDO & MASAMUNE in Ann. Rep. Taihoku Bot. Gard. II. p. 7 (1932) (Gen. Pl. Formos. I.). SUZUKI in MASAMUNE, Short Fl. Formos. p. 39 (1936). KANEHIRA, Formos. Trees ed. 2, p. 76, f. 32 (1936).

Salix Kusanoi HAYATA, Gen. Ind. Fl. Formos. p. 73 (1917). MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1125 (1925).— SASAKI, List Pl. Formos. p. 142 (1928).— KUDO & MASAMUNE in Ann. Rep. Taihoku Bot. Gard. II. p. 6 (1932) (Gen. Pl. Formos. I.).— SUZUKI in MASA-MUNE, Short Fl. Formos. p. 39 (1936).

Arbor dicitur mediocris. *Ramuli* hornotini in sicco fuligineo-fuscis,

plerumque pilis brevibus directione variis tum cinereis tum ferrugineis superne densissime inferne plus minusve laxius velutini; novelli florendi tempore sericei; annotini fusci glabri 2.0–4.5 mm crassi ad nodos torulosi. Cicatrices foliorum fere luniformes; amentorum et ramulorum late orbiculares, concavae, transverse 2.5–4.0 mm longitudine 2.0–3.0 mm latae, margine cortice ramuli colliformiter prominente (latere superiore altius) marginatae. *Gemmae* ovatae apice obtusae atrofuscae 3.5–5.0 mm longae; perula latere dorsali sub lente puberula ceterum glabra, ventrali libera, marginibus imbricatis. *Cataphyllum* primum sterilis ramuli perulaceum adaxiale late orbiculare, apice saepe bifidum coriaceum brunneumque, extus basi puberulum, intus glaberrimum, circiter 6 mm longum 6–8 mm latum; secundum abaxiale late obovatum apice plerumque bifidum, intus glaberrimum extus sericeum, integerrimum 5–11 mm longum, in expansione 5–10 mm latum; tertium laterali-adaxiale vel fere adaxiale, obovatum intus glaberrimum extus sericeum 8–11 mm longum 5–7 mm latum; cetera obovata ad elliptica, apice rotundata et ad summum mucronulata, basi obtusa, margine fere integerrima vel minutissime crenato-denticulata, 7–13 mm longa, 4.5–8 mm lata, utroque latere 9–11-nervatula, supra undique vel suprema parte excepta minute sericeo-pubescentia, subtus dense adpresseque villoso-sericea, pilis marginem superantibus, superiora majora et margine serrulata. *Folia adulta* supra non stomatifera, chartaceo-coriacea in sicco supra fuscescentia infra pallide luteo-brunnea, *superiora* elliptico-oblonga vel lanceolato-oblonga apice longe acuminata, basi cordata vel subaurita, ad vel paullo infra medium latiora, margine crenato-serrulata, dentibus 4–6 pro 1 cm, supra praeter costam glaberrima vel parce adpresse pilosa, subtus primo griseo- et fulvo-sericea, demum glabrescentia, 8.2–9.8 cm longa 3.0–4.0 cm lata; *costa* supra sericea in sicco plana circa basin impressa, subtus prominente griseo- et fulvo-sericea; nervis primariis utrinsecus 14–20 supra in sicco planis infra elevatis leviter arcuatis sub angulis 50°–60° divergentibus ante marginem adscendentibus; secundariis crebris satis tenuibus indistinctisque; intermediis 1–3, quorum superioribus longioribus, primariis fere parallelis. *Folia adulta inferiora* elliptica vel ovato-elliptica apice breviter acuminata basi ut in superioribus 6–7.5 cm longis, 3.2–4.0 cm latis, nervis primariis 10–13 a costa sub angulis 60°–70° divergentibus, ceterum ut in superioribus. *Folia intermedia* ovali-elliptica apice late acuta vel rotundata vel breviter acuminata, basi leviter cordata 3–4 × 1.9–2.7 cm magna. Folia recentissima convoluta utrinque pilis cinereis et fulvis dense velutino-sericea. *Petioli* supra sulcati pubescentes ad 1.8 cm longi eglandulosi. Stipulae non visae. *Amenta* ♂ elongato-cylindrica, praecocia vel

subcoaetanea, 5.5–9.0 cm longa 7–9 mm crassa, sublaxiflora, rhachidibus pubescentibus per totam longitudinem visibilibus, pedunculis 1.2–1.6 cm longis minute cinereo-pubescentibus, cataphyllis caducis 4–5 dispositione ceterisque notis iis sterilium ramulorum plane congruentibus suffultis. *Bracteolae* luteae concolores obovato-oblongae vel obovatae, apice rotundatae, latere involutae ciliatae, intus villosae, extus dimidia inferiore parte pilis crispatis albo-villosae 2.5–3.5 mm longae, in expansione 1.6–2.0 mm latae. *Glandulae* 2 basi connatae et pseudodiscum vulgo 5-lobulatum formantes, parte ventrali crassa lata et brevi, aut truncata aut emarginata vel bifida, 0.4–0.5 mm longa 0.8–0.9 mm lata, dorsali truncato-ovali saepe 3-lobulata, lobulo medio majore 0.5 mm longo 0.8 mm lato. *Stamina* 6–7, filamentis liberis basi pilosis 2.5–4.0 mm longis. *Antherae* ovales et flavae sub anthesi 0.4–0.6 mm longae. *Amenta* ♀ (sub anthesi non visa) tantum deflorata et fructifera visa; deflorata 3.5–5.0 cm longa 0.8 cm crassa; fructifera ad 10 cm longa. Rhachis visibilis et pubescens vel tomentosa. Cataphylla ♀ amentorum caduca, solum superiora visa, obovata apice mucronata vel cuspidato-mucronata, basi cuneato-acuta ad rotundata, sessilia vel breviter petiolata, margine minute et leviter undulata vel fere integerrima, supra sericea vel sub lente minute et parce puberula, subtus undique sericea 11 × 8, 15 × 9, 18 × 12 mm etc. magna. *Bracteolae* dehiscente capsula demum deciduae obovato-ellipticae apice obtusissimae, ceterum ut in ♂. *Glandula* ventralis lata et crassa 0.4–0.5 mm alta 0.8 mm lata, apice truncata interdum leviter emarginata, basin pedicelli semicirculatim cingens. Glandula dorsalis nulla vel minima globosa saepe partita et ventrali connata. *Ovaria* lanceolato-conica apice obtusissima glaberrima; pedicellis glaberrimis circ. 0.8 mm longis; stylis 0.5–0.6 mm longis sat obcompressis. Stigmata brevia emarginata. Capsulae ad 6.5 mm longae basi 2 mm vel ultra crassae.

NOM. JAP. *Suisya-yanagi* HAYATA, Gen. Ind. Fl. Formos. p. 73 (1917).— *Kusano-yanagi* HAYATA, Gen. Ind. Fl. Formos. p. 73 (1917).

HAB. Japonia. *Formosa.*— Prov. **Takao**: Bansyoryô, (S. KUSANO Feb. 1909 [Typus *S. tetraspermae* var. *Kusanoi* HAYATA] in Herb. Univ. Imp. Tokyo.).— Prov. **Taityû**: Suisya, (B. HAYATA st. 29 Apr. 1916 [Typus *S. suishaensis* HAYATA] in Herb. Univ. Imp. Tokyo.— R. KANEHIRA & S. SASAKI n. 10 ♂ & ♀ 11 Mart. 1918 in Herb. Univ. Imp. Tokyo.— I. TANAKA st. Jun. 1918 in Herb. Univ. Taihoku.— S. KITAMURA fr. & ♂ 3 Mart. 1932 in Herb. Univ. Imp. Kyoto.); insula Syusi in lac. Zitugetutan, (KUDO & SASAKI n. 15540 st. 21 Sept. 1929 in Herb. A. KIMURA); in paludibus insulae innatae in Nichigetsutan prope Mukaiyama, (KUDO &

SASAKI n. 15637 st. 22 Sept. 1929 in Herb. Univ. Imp. Taihoku.); inter
Sekiin et Bokkitsu ad Nichigetsutan, (KUDO & SASAKI n. 15275 st. 18
Sept. 1929 in Herb. Univ. Imp. Taihoku.).

54) **Turanga** KIMURA gen. nov. Salicacearum.

Syn. *Populus* sect. *Turanga* BUNGE in Mém. Sav. Étr. Acad. Sci. St. Pétersbourg VII.
p. 498 (1851) (A. LEMANN Rel. Bot. p. 322).- C. K. SCHNEIDER. Ill. Handb. Laubholzk. I.
p. 5 (1904); in SARGENT, Pl. Wilson. III. p. 29 (1916).- SEEMEN in ASCHERSON & GRAEBNER,
Syn. Mitteleurop. Fl. p. 15 (1908). - GOMBOCZ in Ma'h. Termész. Közl. XXX. p. 67 (1908)
(Monogr. Gen. Populi).

Populus subgen. *Turanga* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr.
Monogr. Inéd. Populus p. 13).— KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 221 (1936).

Balsamiflua GRIFFITH, Notul. Pl. Asiat. IV. p. 382 1854; Icon. Pl. Asiat. IV. t. 526
(1854).

Arbores. Ramuli gemmis terminalibus carentes, i. e. *ramificatio sympo-
dialis*[1] . Periderma ex hypodermate oriundum, parietibus cellularum
aequaliter tenuibus, scleroblastis instructum (an semper?). Scleroblasti in
cortice interiore non evoluti. Fasciculi fibrarum mechanicarum in regione
circummedullari adsunt. Gemmae 1 2-perulatae, haud vel paullo viscosae.
Folia alterna satis coriacea, polymorpha, isolateralia, utrinque cinereo-glauca
et stomatibus numero aequalibus praedita, basi glandulis vix vel haud
prominentibus munita; petioli plus minusve teretes. Flores dioici in racemis
amentiformibus dispositi, foliis praecociores. Bracteolae caducae dentato-
incisae, glabrae vel breviter ciliatae. Flores ♂ pedicellati; staminibus
numerosis in perigonio disciformi multidentato insertis; filamentis liberis;
antheris longis apice apiculatis. Flores ♀ pedicellati; perigonio *deciduo*
profunde multidentato vel ad basin usque laciniato. Ovarium sessile
uniloculare, carpophyllis 3. Placentae parietales 3, lineares longae.
Stigmata 3 magna decidua stylosa. Ovula ascendentia anatropa in quaque
placenta numerosa. Capsula 3-valvis. Semina numerosa parva, coma densa
e pilis longissimis numerosis ex epidermide placentae[2] (haud funiculi)
oriundis cincta. Testa membranacea. Albumen nullum. Embryo ortho-
tropus. - Typus generis: *Turanga diversifolia* (SCHRENK) KIMURA.

Cum genere *Populo* multa communia habet, sed ramificatione sympo-

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[1] Teste ipso et REHDER in litt.- Hic gratias quam maximas ago ill. Prof. A. REHDER, qui
rogatu meo opinionem confirmavit meam de modo ramificationis in hoc genere ad multa
specimina in herbario Arboreti Arnoldiani conservata. Mihi benigniter scripsit in litteris:
"On our numerous specimens of *Populus euphratica*, terminal buds are not developed and
therefore the branching is sympodial. The same is the case in *P. pruinosa*".

[2] Character hic non est proprius huic generi, sed communis omnibus Salicaceis. Cfr.
etiam TAKEDA, On the Coma or Hairy Tuft of the Seed of Willows, Bot. Mag. Tokyo, L.
p. 283-289 (1936).

diali, cortice interiore scleroblastis carente, foliis isolateralibus, perigoniis deciduis profunde vel ad basin usque dentato-laciniatis generice distinguendum. Species fere 10 inter se evidenter valde affines; Hispaniae, Africae borealis et orientalis, Asiae occidentalis atque meridionalis, Sinae borealis et Mongoliae incolae. Genus in sectiones duas dividitur: —

Sect. 1. Euphraticae (Dode) Kimura comb. nov.

Syn. Groupe *Euphratica* Dode in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus p. 14).

Ряд *Euphraticae* Komarov, Fl. Union. Rer. Sov. Soc. V. p. 221 (1936).

Huc pertinent: *Turanga euphratica, T. diversifolia, T. Litwinowiana, T. Ariana, T. Mauritanica, T. Bonnetiana, T. ilicifolia, T. illicitana;* verisimiliter etiam fossiles *T. mutabilis, T. Berggreni, T. hyperborea, T. Kansaseana, T. Gaudini.*

Sect. 2. Pruinosae (Dode) Kimura comb. nov.

Syn. Groupe *Pruinosa* Dode in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus p. 14.

Ряд *Pruinosae* Komarov, Fl. Union. Rer. Sov. Soc. V. p. 223 (1936).

Huc pertinent *Turanga pruinosa, T. glaucicomans* et verisimiliter fossilis *T. retusa.*

55) Turanga euphratica (Olivier) Kimura comb. nov.

Syn. *Populus euphratica* Olivier, Voy. Emp. Othoman. III. p. 449, fig. 45-46 (1807). — Wesmael in De Candolle, Prodr. XVI. 2, p. 326 (1868); in Mém. Soc. Sci. Hainaut III. (1869) (Monogr. Populus, p. 51, t. 10-13).— Brandis, Forest Fl. North-West & Centr. Ind. p. 474, t. 63 (1874); Indian Trees, p. 639, fig. 198 (1906).— Boissier, Fl. Orient. IV. p. 1194 1879). - Regel in Act. Hort. Petrop. VI. 2, p. 473 (1880). - Trautvetter in Act. Hort. Petrop. IX. 1, p. 190 (1884) (Incr. Fl. Phaenogam. Ross. III.). - Hooker f., Fl. Brit. Ind. V. p. 638 (1888).— Dippel, Handb. Laubholzk. II. p. 196 (1892).— Koehne, Deutsche Dendrol. p. 81 (1893). - ? Burkill in Jour. Linn. Soc. XXVI. p. 536 (1899) (Forbes & Hemsley, Ind. Fl. Sin. II.). Schneider, Ill. Handb. Laubholzk. I. p. 5 (1904); in Sargent, Pl. Wilson. III. p. 30 (1916). - Dode in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 16).— Gombocz in Math. Termész. Közl. XXX. p. 68 (1908) (Monogr. Gen. Populi). - Seemen in Ascherson & Graebner, Syn. Mitteleurop. Fl. p. 16 (1908).— O. & B. Fedtschenko, Consp. Fl. Turkest. VI. p. 330 (1916).— Krause in Fedde, Rep. Sp. Nov. Reg. Veg. XXVIII. p. 79 (1930) (Beiträge z. Fl. Kleinasiens. V). Görz in Fedde, Rep. Sp. Nov. Reg. Veg. XXXVI. p. 20 (1934) (Sched. ad fasc. III. Salicac. Asiatic.).— Komarov, Fl. Union. Rer. Sov. Soc. V. p. 222 (1936).

Populus euphratica var. *typica* Gombocz in Math. Termész. Közl. XXX. p. 71 (1908) (Monogr. Gen. Populi).

Populus biformis Rafinesque, Alsogr. Americ. p. 43 (1838).

Populus euphratensis in Gard. Chronic. 1849, p. 806.

Balsamiflua deltoides Griffith, Icon. Pl. Asiat. IV. t. 526 (1854) icon videtur partim mala; Notul. Pl. Asiat. IV. p. 382 (1854 .

HAB. ex Asia occidentali et meridionali usque ad Sinam borealem et Mongoliam.

56) Turanga diversifolia (SCHRENK) KIMURA comb. nov.

Syn. *Populus diversifolia* SCHRENK in Bull. Acad. Sci. St. Pétersbourg X. p. 253 (1842); in FISCHER & MEYER, Enum. Pl. Nov. Schrenk. II. p. 15 (1842).— LEDEBOUR, Fl. Ross. III. p. 627 (1850).— TRAUTVETTER, Pl. Imag. Fl. Russ. p. 23, t. 16 (1844).— DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 15).— KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 221 (1936).

Populus euphratica var. *diversifolia* GOMBOCZ in Math. Termész. Közl. XXX. p. 71 (1908) (Monogr. Gen. Populi).

Populus euphratica B. FEDTSCHENKO, Rast. Turkest. p. 292 (1915) fide KOMAROV.

HAB. in regione Altaica.

57) Turanga Litwinowiana (DODE) KIMURA comb. nov.

Syn. *Populus Litwinowiana* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 17); in FEDDE, Rep. Sp. Nov. Reg. Veg. III. p. 158 (1906).- KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 222 (1936).

Populus euphratica var. *typica* f. *hirta* (LITWINOW) GOMBOCZ in Math. Termész. Közl. XXX. p. 71 (1908) (Monogr. Gen. Populi).

HAB. in Asia centrali.

58) Turanga Ariana (DODE) KIMURA comb. nov.

Syn. *Populus Ariana* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 16); in FEDDE, Rep. Sp. Nov. Reg. Veg. III. p. 157 (1906).— KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 222 (1936).

Populus euphratica LIPSKY, Ljesn. rast. v Turkest. p. 17 (1911) fide KOMAROV.

HAB. in Persia.

59) Turanga Mauritanica (DODE) KIMURA comb. nov.

Syn. *Populus Mauritanica* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 16); in FEDDE, Rep. Sp. Nov. Reg. Veg. III. p. 157 (1906).

HAB. in Africa boreali.

60) Turanga Bonnetiana (DODE) KIMURA comb. nov.

Syn. *Populus Bonnetiana* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus, p. 16); in FEDDE, Rep. Sp. Nov. Reg. Veg. III. p. 157 (1906).

Populus euphratica var. *typica* f. *Bonnetiana* (DODE) GOMBOCZ in Math. Termész. Közl. XXX. p. 72 (1908) (Monogr. Gen. Populi).

HAB. in Africa boreali.

61) Turanga ilicifolia (ENGLER) KIMURA comb. nov.

Syn. *Celtis ilicifolia* ENGLER, Pflanzenwelt Ost-Afrikas p. 160 (1895).

Populus euphratica OLIVIER subsp. *Denhardtiorum* ENGLER in Notizbl. Kön. Bot. Gart. Mus. Berlin II. p. 218 (1898).

Populus euphratica OLIVIER var., *Denhardtiorum* GOMBOCZ in Math. Termész. Közl. XXX. p. 72 (1908) (Monogr. Gen. Populi).

HAB. in Africa tropica, "Ufer des Tsavo und Athi-Flusses".
Propter fructus magnos species propriam efficere videtur.

62) **Turanga illicitana** (DODE) KIMURA comb. nov.

Syn. *Populus illicitana* DODE in Bull. Soc. Dendrol. France 1908 p. 163, cum fig.--
ASCHERSON in Ber. Deutsch. Bot. Ges. XXVI. p. 358-360 (1908).

HAB. in vicinitate urbis Illices, in Tarraconense Hispaniae provincia.

63) **Turanga pruinosa** (SCHRENK) KIMURA comb. nov.

Syn. *Populus pruinosa* SCHRENK in Bull. Acad. Sci. St. Pétersbourg III. p. 210 (1845).
LEDEBOUR, Fl. Ross. III. p. 628 (1850). WESMAEL in DE CANDOLLE, Prodr. XVI. 2, p.
327 (1868); in Mém. Soc. Sci. Hainaut III. (1869) 'Monogr. Populus, p. 56, t. 14).-- DODE
in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr. Monogr. Inéd. Populus p. 18).— GOM-
BOCZ in Math. Termész. Közl. XXX. p. 72 (1908) (Monogr. Gen. Populi).-- SCHNEIDER in
SARGENT, Pl. Wilson. III. p. 30 (1916).- O. & B. FEDTSCHENKO, Consp. Fl. Turkest. VI. p.
331 (1916). - KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 223 (1936).
Populus euphratica WESMAEL in Bull. Soc. Roy. Belg. XXVI. p. 375 (1887) fide GOMBOCZ.

HAB. in Sibiria austro-occidentali.

64) × **Turanga glaucicomans** (DODE) KIMURA comb. nov.

= *T. Ariana* × *T. pruinosa* sec. KOMAROV.

Syn. *Populus glaucicomans* DODE in Mém. Soc. Hist. Nat. Autun, XVIII. (1905) (Extr.
Monogr. Inéd. Populus p. 18). KOMAROV, Fl. Union. Rer. Sov. Soc. V. p. 223 (1936).

HAB. in Turchestania.

65) **Turanga Berggreni** (HEER) KIMURA comb. nov. (fossilis).

Syn. *Populus Berggreni* HEER, Fl. Foss. Arct. III. 2, p. 106, t. XXIX. f. 1-5 (1874).—
GOMBOCZ in Math. Termész. Közl. XXX. p. 167 1908).-

66) **Turanga hyperborea** (HEER) KIMURA comb. nov. (fossilis).

Syn. *Populus hyperborea* HEER, Fl. Foss. Arct. III. 2, p. 106, t. XXIX. f. 6-9, t. XXVII.
f. 8 d., t. XXX. f. 2 b (1874).-- GOMBOCZ in Math. Termész. Közl. XXX. p. 167 (1908).

67) **Turanga Kansaseana** (LESQUEREUX) KIMURA comb. nov. (fossilis).

Syn. *Populus Kansaseana* LESQUEREUX in POWELL, Monogr. U. S. Geol. Survey, XVII.
p. 42, t. XVII. f. 1-7 (1892) (The Flora of the Dakota Group). GOMBOCZ in Math. Termész.
Közl. XXX. p. 167 (1908).

68) **Turanga Gaudini** (FISCHER-OOSTER) KIMURA comb. nov. (fossilis).

Syn. *Populus Gaudini* FISCHER-OOSTER ex HEER, Fl. Tert. Helv. II. p. 24, t. LXIV.
(1856). SCHIMPER, Traité Paléont. Végét. II. p. 699 (1870-1872).— GOMBOCZ in Math.
Termész. Közl XXX. p. 168 (1908).

69) **Turanga mutabilis** (HEER) KIMURA comb. nov. (fossilis).

Syn. *Populus mutabilis* HEER, Fl. Tert. Helv. II. p. 19 (1856).-- SCHIMPER, Traité Paléont.
Végét. II. p. 694 (1870 1872).— GOMBOCZ in Math. Termész. Közl. XXX. p. 169 (1908).
Populus ovalis A. BRAUN in BUCKLAND, Geolog. & Mineralog.

Populus ovalifolia A. BRAUN in BRONNS Jahrb. p. 169 (1845).
Salix lancifolia A. BRAUN olim UNGER, Gen. & Sp. Pl. p. 419.
Laurus dermatophyllon O. WEBER in Palaeontogr. II. p. 182, t. XIX. f. 13 (1852).
Populus Aeoli A. BRAUN in BRÜCKMANN, Fl. Oening. Foss. p. 230 (1850).
Ficus pannonica ETTINGSHAUSEN, Foss. Fl. v. Tokay p. 26, t. I. f. 9 (1854).
Quercus ovalis GOEPPERT, Foss. Fl. v. Schossnitz t. VI. f. 6 (1855).

70) **Turanga retusa** (HEER) KIMURA comb. nov. (fossilis).

Syn. *Populus retusa* HEER, Fl. Foss. Arct. IV. p. 69, t. XIV. f. 6-7 (1876).— GOMBOCZ in Math. Termész. Közl. XXX. p. 172 (1908).

71) **Chosenia macrolepis** (TURCZANINOW) KOMAROV in JACZEWSKI, Mél. Bot. Borodine Jubil. LXXX. p. 281 (1927).

mstr. **adenantha** KIMURA mstr. nov. (Fig. 1 & Tab. XII).
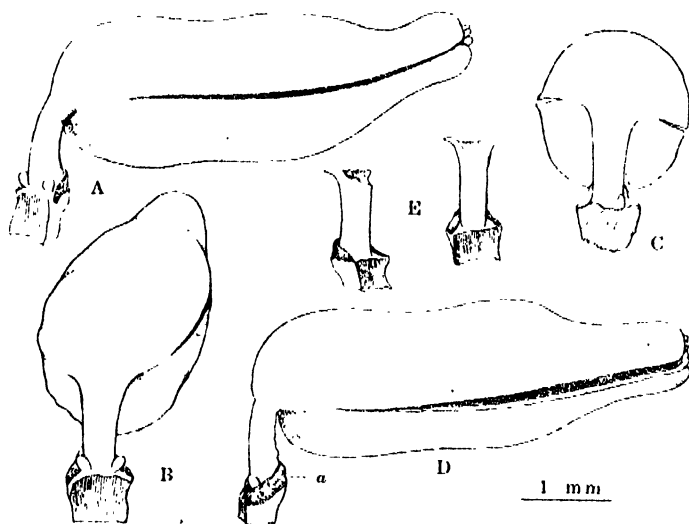
Fig. 1. *Chosenia macrolepis* (TURCZ.) KOMAROV mstr. *adenantha* KIMURA.
A Capsula cum glandulis duabus aequiformibus a latere. *B* Eadem a ventre.
C Capsula cum glandula una a ventre. *D* Eadem a latere; *a* Cicatrix bracteolae. *E* Stipites glandulis nullis, resectis capsulis.

Discrepat a typica et distinctissima capsulis plerisque glandulosis.— Glandulae plerumque 2 aequiformes (obovatae, oblongae vel ovatae, 0.2-0.3 mm longae) ad basin stipitis capsulae latere sitae eundem in modum *Toisusu* generis (Fig. 1. *A, B*), saepe tamen quarum una tantum bene evoluta alteraque minima vel plane reducta (Fig. 1. *C, D*), denique utraque toto nulla ut in typica (Fig. 1. *E*). Flores glandulosi et eglandulosi in eodem amento.

Quoad figuram, magnitudinem coloremque omnium ceterarum partium i. e. capsularum cum stipitibus, cicatricum bracteolarum, amentorum fructiferorum, foliorum et ramulorum optime cum typica congruit. Nulla signa inveniri possunt mixtrae cum *Toisusu* vel ceteris *Salicaceis*. Haec monstrositas, quam pro atavismo habeo, genus *Choseniam* e proxime affini *Toisusu* genere glandulis suppressis, ut opinor, transformatum monstrat; quare haec genera in unam eandemque tribum *Chosenieae* impono (vide infra).

HAB. in Manchuria: Jalatun, (P. H. & J. H. DORSETT n. 3481 [typus mstr.] fructif. Junio 1925, in Herb. Hort. Bot. Noveboracensis); Barun, (P. H. & J. H. DORSETT n. 3547 fructif. 30 Junio 1925 in Herb. Hort. Bot. Noveboracensis).

72) SYSTEMA NOVUM SALICACEARUM, SIVE EMENDATIONES ET ADDITAMENTA AD SYSTEMA SALICACEARUM ANNO 1928 AB AUCTORE PROPOSITUM.

SYNOPSIS DISPOSITIONIS.

Subfam. 1. *Populoideae* KIMURA.
　Tribus 1. *Populeae* NAKAI.
　　　　　Genus *Populus* LINNAEUS.
　Tribus 2. *Turangeae* KIMURA.
　　　　　Genus *Turanga* KIMURA.
Subfam. 2. *Salicoideae* KIMURA.
　Tribus 1. *Chosenieae* KIMURA.
　　Subtrib. 1. *Toisusinae* KIMURA.
　　　　　Genus *Toisusu* KIMURA.
　　Subtrib. 2. *Choseniinae* KIMURA.
　　　　　Genus *Chosenia* NAKAI.
　Tribus 2. *Saliceae* KIMURA.
　　　　　Genus *Salix* LINNAEUS.
　　　　　　Subgen. 1. *Protitea* KIMURA.
　　　　　　Subgen. 2. *Euitea* KIMURA.

Subfam. **Populoideae** KIMURA in Tokyo Bot. Mag. XLII. p. 290 (1928) emend. KIMURA.— Arbores. Ramuli saepius gemmis terminalibus instructi, i. e. ramificatio monopodialis (*Populus*), rarius carentes, i. e. ramificatio sympodialis (*Turanga*). Periderma ex hypodermate oriundum, parietibus cellularum aequaliter tenuibus. Scleroblasti in cortice interiore saepius evoluti. Fasciculi fibrarum mechanicarum in regione circummedullari adsunt. Gemmae uni- vel bi- vel pluriperulatae, perulis imbricatis. Folia alterna, petiolis haud raro a latere compressis, saepius lata, pennivenia et basi fere 3-nervia, integerrima dentata vel lobata, in eodem ramulo saepe

valde heteromorpha. Amenta praecocia, saepius pendula, floribus saepe breviter pedicellatis. Bracteolae caducae saepe in stipitem contractae, apice dilatatae integrae dentatae vel ciliato-fimbriatae. Perigonium (=discus) carnosulum vel membranaceum, cyathiforme, saepe obliquum, margine integrum, crenatum, sinuatum, dentatum vel profunde lobatum, saepius persistens (*Populus*), rarius deciduum (*Turanga*). Fl. ☿: Stamina 4 — numerosa, filamentis liberis filiformibus; antherae saepe majusculae. Grana pollinis anemophila, sphaeroidea vel plus minusve irregularia, exosporio tenui acolpato laevi vix reticulato. Fl. ♀: Ovarium intra perigonium sessile, 1-loculare, carpophyllis saepius 2, raro 3-4; placentae parietales saepius 2, raro 3-4; styli breves vel longiusculi. Stigmata 2-4 integra vel lobata. Ovula in quaque placenta numerosa, 2—∞-seriata, anatropa, crassinucellata, integumentis 1-2. Cellulae parietales in apice nucelli longitudine 2-seriatae. Fertilisatio aporogama[1].— Typus: *Populus* Linnaeus.

Tribus 1. **Populeae** Nakai[2].— Ramuli gemmis terminalibus instructi (i. e. ramificatio monopodialis). Scleroblasti in cortice interiore fere evoluti. Gemmae pluriperulatae. Perigonium persistens. Carpo-phylla saepius 2, raro 3-4. Ceterae notae subfamiliae.— Huc pertinet *Populus* Linnaeus (Typus tribus).

Tribus 2. **Turangeae** Kimura trib. nov.- Ramuli gemmis terminalibus carentes (i. e. ramificatio sympodialis). Scleroblasti in cortice interiore non evoluti. Gemmae 2- non numquam 1-perulatae[3]. Folia poly-morpha isolateralia. Perigonium deciduum. Carpophylla 3. Ceterae notae subfamiliae.-- Huc pertinet *Turanga* Kimura (Typus tribus).

Subfam. **Salicoideae** Kimura in Tokyo Bot. Mag. XLII. p. 290 (1928).- Arbores vel frutices. Ramuli gemmis terminalibus carentes, i. e. ramificatio sympodialis. Periderma ex epidermide oriundum, parietibus exterioribus cellurarum crassioribus[4]. Scleroblasti in cortice interiore fere nulli. Fasciculi fibrarum mechanicarum in regione circummedullari desunt. Gemmae uniperulatae; perulis originaliter e prophyllis *a* et *β* compositis, aliis in unam calyptriformem perfecte connatis, aliis dorso tantum connatis ventre autem liberis et imbricatis. Folia alterna rarius opposita, petiolis

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[1] Graf, J. Beiträge zur Kenntnis der Gattung Populus. Beih. Bot. Centralbl. XXXVIII, p. 405-454, t. 10, 11 (1921).

[2] *Salicaceae* trib. I, Populeae Nakai, Fl. Sylv. Korea. XVIII. p. 55, 183 (1930).

[3] Eundem in modum quarundam specierum *Salicis* subgeneris *Protiteae*.

[4] Teste ipso, quoad genera *Choseniam* et *Toisusu*.

plerisque brevibus, penninervia, integerrima vel serrulata. Amenta praecocia, coaetanea vel serotina, erecta, declinata vel pendula. Bracteolae integerrimae vel rarius dentatae. Perigonium glanduliforme vel nullum. Fl. ☿: Stamina (1–)2–5(–20), filamentis liberis vel connatis. Grana pollinis saepius entomophila rarius anemophila, tricolpata, exosporio distincte reticulato. Fl. ♀: Ovarium uniloculare, carpophyllis 2. Placentae 2 parietales vel basilari-parietales. Styli 2, saepius in unum connati, rarius liberi. Stigmata 2, integra vel bifida. Ovula in quaque placenta 2–10, 2—∞-seriata, anatropa, crassinucellata, integumento 1. Cellulae parietales in apice nucelli longitudine 1–2-seriatae. Tubus pollinis per micropylen oosphaeram attingens, i. e. fertilisatio porogama[1].—Typus: *Salix* LINNAEUS.

  Tribus 1. **Chosenieae** KIMURA in Tokyo Bot. Mag. XLII. p. 290 (1928) emend. KIMURA.— Gemmae uniperulatae, perulis ventre liberis, marginibus imbricatis. Cataphyllum primum adaxiale, secundum abaxiale[2]. Amenta cylindrica sub anthesi pendula[2] (Tab. XIII & XIV). Bracteolae concolores, in ♀ caducae. Fl. ☿ : Glandulae adsunt vel nullae. Stamina 5 vel plura, filamentis liberis. Grana pollinis entomophila vel anemophila. Fl. ♀ : Glandulae adsunt vel nullae. Ovarium stipitatum. Styli 2 liberi vel ad medium usque connati.. Stigmata in laciniis duabus elongatis angustisque partita, cum dimidio superiore styli decidua. Placentae basilari-parietales. Ovula in quaque placenta 2, biseriata. Chromosomata 2n = 38[2]. Ceterae notae subfamiliae.— Typus: *Chosenia* NAKAI.

    Subtribus 1. **Toisusinae** KIMURA subtrib. nov.— Fl. ☿ : Glandulae 2 ventralis et dorsalis, utraque saepius emarginata vel 2–3-partita. Stamina 5–10. Grana pollinis entomophila. Fl. ♀ : Glandulae 2 aequiformes globosae vel oblongae lateraliter dispositae. Ceterae notae tribus.— Huc pertinet *Toisusu* KIMURA (Typus subtribus).

    Subtribus 2. **Choseniinae** KIMURA subtrib. nov.— Fl. ☿ : Glandulae nullae. Stamina semper 5. Grana pollinis anemophila, notis autem morphologicis verae *Salicis*[2]. Fl. ♀ : Glandulae nullae. Ceterae notae tribus.— Huc pertinet *Chosenia*[2] NAKAI (Typus subtribus).

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[1] CHAMBERLAIN, C. J., Contributions to the life history of Salix. Bot. Gaz. XXIII. p. 147–179, t. 12–18 (1897). Praeterea ipse etiam in *Chosenia* et ceteris porogamiam observavi (Fig. 2 in pagina sequente).

[2] Teste ipso!

[3] Genus hoc ab *Toisusu* generice differt tantum florum glandulis toto nullis.

Tribus 2. **Saliceae** KIMURA in Tokyo Bot. Mag. XLII. p. 290 (1928) emend. KIMURA (excl. *Toisusu*). Gemmae uniperulatae, perulis ventre nunc connatis (subgen. *Euitea*) nunc liberis et imbricatis (subgen. *Protitea*). Cataphyllum primum adaxiale vel laterali-adaxiale vel laterale, secundum abaxiale vel laterale[1]. Amenta sub anthesi pleraque erecta non numquam declinata. Bracteolae concolores vel discolores, in ♀ persistentes vel deciduae. Fl. ☿ : Glandulae adsunt. Stamina saepius 2–5 vel plura, rarissime 1, filamentis liberis vel connatis. Grana pollinis entomophila. Fl. ♀ : Glandulae adsunt. Ovarium

Fig. 2. Sectio longitudinalis ovuli *Choseniae macrolepidis*, porogamiam monstrans. *a* Sacculus embryonalis. *b* Tubus pollinis in micropyle. × 410.

sessile vel stipitatum. Styli saepius connati. Stigmata integra, emarginata, bifida, persistentia. Placentae basilari-parietales vel parietales. Ovula in quaque placenta 2–10, 2 ∞-seriata. Ceterae notae subfamiliae. Huc pertinet *Salix* LINNAEUS. - Typus: *Salix* LINNAEUS.

Genus **Salix** LINNAEUS, Sp. Pl. ed. 1, p.1015 (1753); Gen. Pl. ed. 5, p. 447 (1754). — Notae tribus.

Subgenus 1. **Protitea** KIMURA in Tokyo Bot. Mag. XLII. p. 290 (1928). Perula gemmae ventre libera, marginibus imbricatis. Cataphyllum primum adaxiale, secundum abaxiale[1]. Bracteolae concolores, in ♀ saepius deciduae, raro persistentes. Fl. ☿ : Glandulae saepius cyathiformes, androecium cingentes, margine undulatae, sinuatae vel plus minus lobulatae. Stamina 5 vel plura, filamentis liberis. Fl. ♀ : Glandulae figura variae; aliae plus minus oblique cyathiformes stipitem ovarii

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[1] Teste ipso!

perfecte cingentes, margine truncatae, undulatae vel lobulatae, aliae in partem ventralem crassam latam interdum irregulariter lobulatam stipitem ovarii semiamplectentem et dorsalem minimam saepe satis reductam partitae. Ovarium plus minus longe stipitatum, stylis brevissimis. Stigmata brevia. Typus: *Salix chaenomeloides* KIMURA.

Subgenus 2. **Euitea** KIMURA in Tokyo Bot. Mag. XLII. p. 290 (1928). Perula gemmae ventre connata calyptriformis. Cataphyllum primum adaxiale vel laterali-adaxiale vel laterale, secundum abaxiale vel laterali-abaxiale vel laterale[1]. Bracteolae concolores vel discolores, in ♀ persistentes vel deciduae. Fl. ☿ : Glandulae 2 ventralis et dorsalis, rarius basi connatae (in formis maxime alpinis atque arcticis nonnullis), vel tantum una dorsalis. Stamina saepius 2, rarissime 1, in sect. *Triandrae* 3, sect. *Pentandrae* (et affin.) 5—plura, filamentis liberis vel connatis. Fl. ♀ : Glandulae 2 ventralis et dorsalis, rarius basi connatae et pseudodiscum quasi formantes (in formis maxime alpinis et ceteris paucis), vel tantum unica dorsalis. Ovarium sessile vel stipitatum. Styli varii. Stigmata varia.

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EXPLICATIO TABULARUM

TAB. XII.

Chosenia macrolepis TURCZ. KOMAROV mstr. *adenantha* KIMURA. Typus.

TAB. XIII.

Fig. 1. *Chosenia macrolepis* (TURCZ.) KOMAROV ♀. Ramulus amentis exacte sub anthesi ornatus. ×0.67. Photogr. 27 Maji 1929.

Fig. 2. *Chosenia macrolepis* (TURCZ.) KOMAROV ☿. Ramulus cum amentis sub anthesi. ×0.87. Photogr. 27 Maji 1929.

TAB. XIV.

Fig. 1. *Toisusu Urbaniana* (SEEMEN) KIMURA ♀. Ramulus cum amentis exacte sub anthesi. ·0.3. Photogr. 27 Maji 1930.

Fig. 2. *Toisusu Urbaniana* (SEEMEN) KIMURA ☿. Ramulus cum amentis sub anthesi. ×0.33. Photogr. 25 Maji 1930.

Omnes figurae ab auctore ipso photographatae in alpina convalle in Japonia media quae japonice *Kamikoti* appellatur.

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[1] Teste ipso!

A. KIMURA : Symbolae Iteologicae VI.

Fig. 1.

Fig. 2.

A. KIMURA : Symbolae Iteologicae VI.

Fig. 1.

Fig. 2.

A. KIMURA: Symbolae Iteologicae VI.

THE NERVOUS SYSTEM OF EARTHWORM (*PHERETIMA COMMUNISSIMA*) IN DIFFERENT AGES

By

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(With Plate XV–XVII and 36 figures)

(Received October 26, 1938)

CONTENTS

I. INTRODUCTION

During my previous work upon the number of ganglion cells and nerve fibers of earthworms, I felt the necessity of investigating further the finer

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structure of the nervous system either by the vital staining or by the silver impregnation method; for only by these methods can the numerical relation between these elements be exactly understood.

In my first investigation I had ascertained that in the typical segment the number of nerve fibers running from a ganglion of the ventral cord through three paired segmental nerves to the body wall amounts altogether to only 2/5 of the number of nerve cells contained in the ganglion concerned, while the nerve fibers passing the neuropile of the interganglia are more than twice as many as the nerve cells in the ganglion. For the precise explanation of these results we must first know the detailed forms of the neurons.

Therefore, I intend in the present work to solve the following problems: (1) How many processes issue from the neuron in the ventral cord and cerebral ganglion? (2) What is the course of nerve fibers traced from the cells inside the ganglia to their peripheral end organs? By applying the intravitam method with methylene blue, I wish to determine the ratio of neurites crossing over to the other side to those which leave the ganglion homolaterally; and also to determine the ratio of nerve fibers running through the interganglia into the consecutive segments to those given off directly from the ganglion concerned. The answer to these problems must naturally have the most intimate relation to the numerical results in my previous work.

The finer internal structure of the nervous system of earthworms has been studied by many predecessors. According to some of them, such as CAJAL ('04) and BOULÉ ('07, '08), who have used the method of silver impregnation, most of the nerve cells of the earthworm are unipolar or multipolar, while only a few are said to be bipolar in shape. On the other hand the paths of the neurons were traced by HALLER ('89, '10), CERFONTAINE ('92), RETZIUS ('92), and KRAWANY ('05), applying either the GOLGI or methylene blue method, and these authors came to the conclusion that motor processes cross more often from one side to the other than the sensory ones and the connection between two consecutive ganglia is partly homolateral, and partly heterolateral. FORTUYN ('20) and PROSSER ('35) made several diagrams based upon the descriptions and drawings of the course of neurons by the above mentioned investigators. From these diagrams we can easily see what great differences exist in their views.

Besides my investigations of the nerve cells in the central nervous system, I have directed special attention to the intermuscular nerve cells,

which are usually found in the peripheral nerve rings and in the muscle layer. DECHANT ('06) and DAWSON ('20) found the intermuscular nerve cells in *Lumbricus*, and explained that these cells may be sensory in nature, probably proprioceptive. In recent time ZYENG ('32) has enumerated the number of them in *Pheretima* and *Eisenia*. As to the intermuscular nerve cells, I have observed chiefly the manner of development of them in young worms, for these cells seem to be an important key to the understanding of the phylogeny of the nervous system. In future investigations I wish to study this problem more in detail with embryos in very early stages. Next let us consider the epithelial sensory cells, which are in close relation to the intermuscular nerve cells. According to numerous investigators, such as CERFONTAINE ('92), LANGDON ('95), RETZIUS ('92), HESSE ('96), and HESS ('23), these epithelial sensory nerve cells bear hairs at their peripheral end and some of the cells arranged in groups of thirty or forty constitute special sense organs. Moreover I shall try to show the relation between the intermuscular and the sensory nerve cells.

Another object of the present paper is to estimate the increase of nerve cells and fibers in the ganglia during the growth from birth to adult age. In this respect I took the ganglia connected with the genital organs into special consideration, the development of which was examined in correlation to that of the nervous system. As for the mode of examination in different stages of growth I used the same method as the school of DONALDSON. On the other hand, as I am much interested by the recent report of TRAGER ('35) on the larvae of *Lucilia* (Fly) stating that the nerve cells differ remarkably from other somatic cells in the manner of increase in size, I have also measured several sorts of cells in the principal parts of the body wall of the earthworm, namely, the sensory nerve cells, the supporting cells, the gland cells, the muscle cells and setae, in order to compare them with the ganglion cells of the nervous system.

II MATERIALS

The materials used in the present investigation belong to *Pheretima communissima* GOTÔ et HATAI, namely the same species as I treated in my previous theses:

First, the standard of growth phases was determined by observing the worms during a period of three years.

The cocoons were collected in early spring from the field where

this species is found in abundance every year, but the exact identification was made later by watching under the microscope the young worms, which had just hatched out. The cocoons of *Pheretima communissima* do not differ essentially from those of *Eisenia* and *Lumbricus* in structure, as seen in Fig. 1, while those of Indian *Pheretima*, according to the description of BAHL ('06), seem to differ from my materials in the
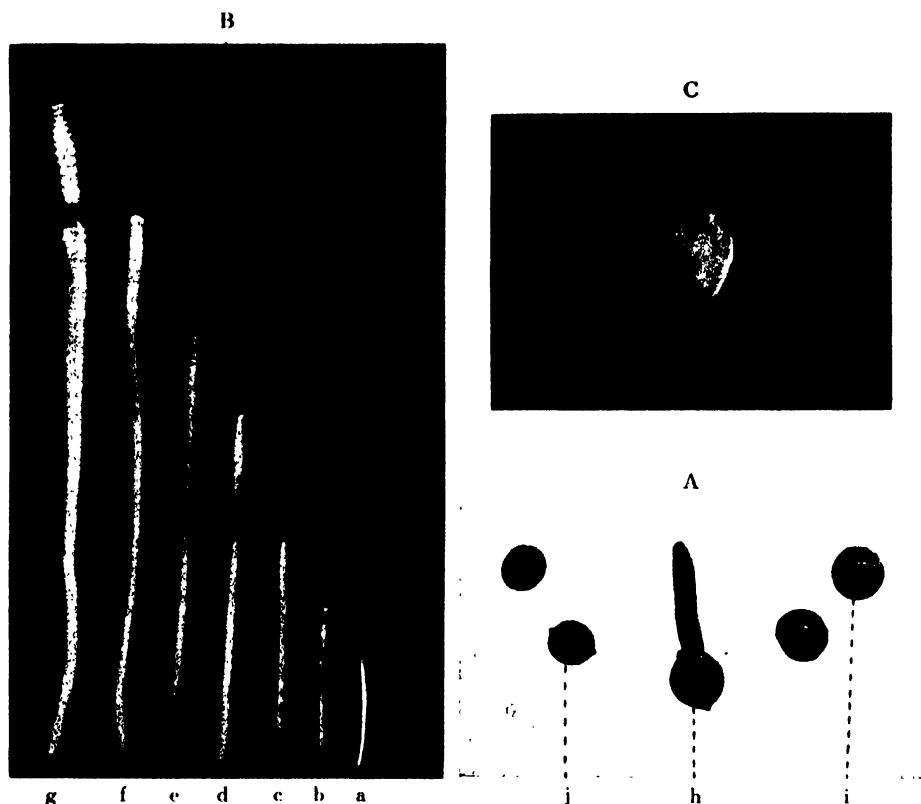

Fig. 1. A) The cocoons and the manner of hatching (h), i) fertilized cocoons, j) unfertilized cocoons; ×2. B) The worms in various stages of growth from birth to adult. a) newly born, b) 3 weeks, c) 6 weeks, d) 9 weeks, e) 12 weeks. f) 15 weeks, g) full grown; ×½. C) The young embryo; ×50.

following several points, although they belong to the same genus. In the Indian *Pheretima* the cocoons are transparent and pale yellow or alight olive-green in colour; in form ovoid, measuring about 1.5 to 2 mm by 1.8 to 2.4 mm, while in the Japanese species they are said to be spherical,

about 4 mm by 4 mm in diameter (Fig. 1, A), not transparent and yellowish brown in colour. In a cocoon of *P. communissima* usually only one embryo, exceptionally two, are found. Fig. 1 represents by photographs the cocoons and the hatching worms.

Newly hatched worms were isolated from the others, and kept in small bowls, which were covered by wire netting to prevent the escape of the worms. The bowls were filled with moist soil and green moss.

TABLE I

The data of various elements during the growth periods of *Pheretima communissima*. (Average of 30 specimens.)

Weeks after hatching		Body-weight in grams	Body-length in millimetres	Diameter in mm.	No. of setae (in XXX)	No. of segments	General remarks	Room temperature
Newly hatching		0.036±0.00045	28±0.042	1.8	60	98	One embryo in each cocoon	13°C
April	I	0.063±0.00052	32±0.052	2.1	,,	100		13°C
	II	0.112±0.0024	38±0.057	2.4	,,	101	Ovary & testis developing	14°C
	III	0.169±0.0048	42±0.069	2.8	,,	102		15°C
	IV	0.213±0.0062	51±0.071	3.2	,,	103	Sperm-reservoir developing	15.6°C
	V	0.370±0.0083	58±0.084	3.5	,,	103	Appearance of male-pores	16°C
May	VI	0.569±0.0097	72±0.089	4.1	,,	104	Appearance of pores of spermatheca	15.5°C
	VII	0.725±0.0103	76±0.092	4.5	,,	105	Developing prostate gland	17°C
	VIII	0.995±0.0016	80±0.097	4.8	,,	106	Appearance of female-pores	17.7°C
	IX	1.75 ±0.0225	94±0.102	5.1	,,	107		18°C
June	X	2.458±0.034	101±0.113	5.5	,,	107	Formation of clitellum	19.5°C
	XI	3.541±0.031	120±0.134	6.0	,,	108		20.1°C
	XII	4.650±0.037	136±0.27	6.2	,,	108		20.9°C
	XIII	5.655±0.043	150±0.37	7.1	,,	108	Cocoons-formation	22.7°C
July	XIV	6.757±0.045	168±0.54	7.5	,,	108		22.6°C
	XV	7.400±0.041	171±0.33	7.8	,,	109		23.2°C
Adult		7.51	171	7.8	,,	109		

Owing to the growth of the worms the bowls had to be replaced by larger ones. During the development the culture soil and moss were renewed each week, when the observation was made to record body weight, body length, diameter of the body, number of setae and segments, etc. These results are given in Table I; in which also the room temperature is indicated in the last column. Since the growth rate of worms is greatly dependent upon the nutrition, temperature, and moisture, one must be very careful to keep the culture soil fresh and moist.

Several years ago T. HINO ('29) studied the growth of the present species, *Pheretima communissima*, but his data do not coincide perfectly with mine in details, as one can see easily by comparing his and my results. As Table I shows, immediately after hatching the earthworm is on the average 28 mm in length, 1.2 mm in breadth and 0.036 grams in body weight, while fourteen weeks after hatching under favourable conditions, it measures 176 mm in length, 7.8 mm in transverse diameter and 7.51 grams in body weight and is at that time to be considered as fully matured. Fig. 1 (B) is a photograph showing the size of the worm at each of the growing periods, and the curves in Chart I indicate the growth rate with respect to length, breadth, and body weight. These curves correspond with the growth curves of animals in general. In addition, to compare with the development of the nervous system, I have observed the growth of some special organs, such as the ducts of the spermatheca, the prostate gland, the sperm-reservoir, the testis, the ovary, the caecum. In the newly hatched worms these are already recognizable as primordial forms under the microscope. Furthermore I examined the number of the setae (in XXXth segment) and of the segments in the newly hatched worms and found that they are almost
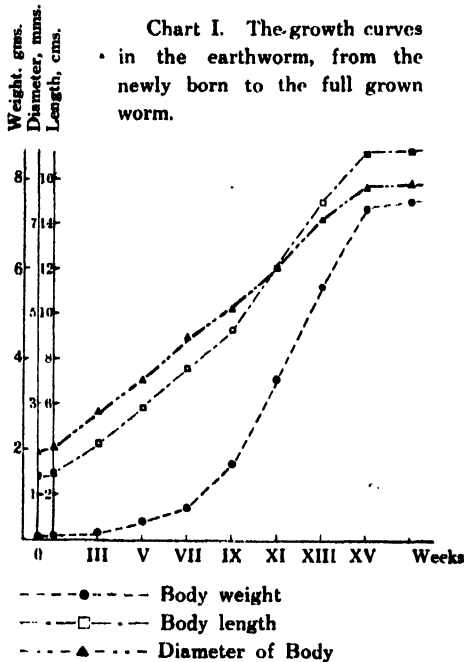
Chart I. The growth curves in the earthworm, from the newly born to the full grown worm.

- - - -●- - - - Body weight
-- -·-□— -·— Body length
- · · -▲ - · · - Diameter of Body

the same as in the adult worm.

According to HINO's observation, the velocity of growth is slower than I found in my specimens, and the difference of growth rate between his field and laboratory specimens is opposite to my results, as in my cases the cultured specimens grew more rapidly than the field ones. In my experiments the clitellum of the rapidly growing worm appeared under favourable circumstances in 10 weeks (9. June), while in HINO's work it took as much as 14 weeks (about the middle in July). On the average, 12 weeks passed after hatching before the clitellum made its appearance (in my observations). In three weeks after the formation of the clitellum, cocoons were produced without copulation by each worm kept in a bowl isolated from any of the others. So far as I am aware, such cocoons have never been described. These cocoons were evidently not fertilized, as they did not develop into embryos the next spring. As seen in Fig. I (A), the non-fertilized cocoons are very small, being only 0.02 grams in weight, instead of 0.03 grams which was the average weight of the fertilized ones that I measured.

In the above mentioned standard of growth, the materials cultured in bowls or collected in the field were employed in this study.

III. TECHNIQUE

The methods employed in the present work may generally be classified into two, one of which is the vital staining with methylene blue in order to see clearly the form of the neurons, and to trace the nerve fibers; while the other is the haematoxylin staining which I used in my previous work in the enumeration of the nervous elements. As for the former, I found that it was not desirable to use the simple methylene blue solution, but that the rongalit white (reduced form of this chemical), which has been used by many workers for this object, gives much better results. With the solution of this chemical either injection or submersion method was employed according to the object of my observation.

A) *Vital staining with methylene blue.*

1) Preparation of the standard solution. — As is well known, the results of this staining are very variable according to the chemicals. Hence, I have used mostly the methylene blue chem. rein, chlorzink frei, from Merck, and the rongalit obtained from Grüber & Co. At first I prepared 0.5 per cent standard solution of methylene blue in distilled water, to which a small quantity of hydrochloric acid was added: 1 or

2 cc. of 25% HCl to 100 cc. of the methylene blue solution. A small quantity of hydrochloric acid when added to the above mentioned solution, seems to make the preparations clearer.

2) Preparation of the rongalit white. — The rongalit white was prepared according to UNNA's method. I dissolved 0.3 g. of rongalit in 10 cc. of the methylene blue solution, and warmed it in a test tube over a small flame, stirring the mixture until the blue colour changed to pale-yellow. After it became cool, I filtered the solution and the filtrate thus obtained makes the standard solution of rongalit white. This solution should be kept in an open test tube covered by a piece of paper. It will keep for about 5 days in spring (at the temperature of 15°-20°C). On the second day after it is made, its staining property is better than immediately after preparation. For staining 1 or 2 drops of the standard solution of rongalit white was added to 30 cc. of the physiological solution. In the physiological experiments on the earthworm, following the recommendation of NOMURA and ISHIKAWA*, who have worked on the physiology of this animal, the 65 per cent diluted Ringer solution was employed throughout the present work.

3) Submersion method. — In this case it is necessary to expose the nervous system for facilitating the penetration of the dye to it. Hence, the worms, being in the physiological solution, were slit along the dorso-median line with fine scissors, and the viscera were removed as much as possible without injuring the nerves. The worms thus operated upon are transferred into a bath of fresh physiological solution with cotton laying at the bottom; then rongalit white solution was added to this bath in proportion of 1 drop to 30 cc. for staining. As the same concentration of rongalit white does not always give an equally successful result owing to several factors, we must search by trials for an adequate concentration of the solution in each case.

Usually after 15 to 30 minutes, the superficial nerve fibers begin to stain and the time needed for the staining depends much upon the temperature. Meanwhile the methylene blue does not reach all of the nerve cells included in the nerve trunk. The preparations were left in the dye for 1 to 4 hours and then, in order to obtain a more complete staining, they were exposed to the action of the air or of the vapour of hydrogen peroxide (3.3% H_2O_2) to cause oxidation. The point when the preparation is ready for fixation is very difficult to determine. Naturally it must

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*Not yet published.

be fixed when the staining reaches its best condition, which is not easy to determine, especially as the nervous elements behave very differently even in the same region of the body. Some of early stained nerve cells may lose their colour, when other ganglion cells begin to take the dye and are not yet blue enough. The most important factors are the environmental temperature and the PH-value of the physiological solution which is liable to be changed by the presence of microorganisms. According to my opinion the proper PH of the solution for the staining is 7.5 and, when it is lower than 7, desirable results are very difficult to attain. When the room temperature is warm, it is sufficient to expose the materials to the vapour of hydrogen peroxide, which seems to accelerate the oxidation of the dye. On the other hand, it is especially necessary to regulate the concentration of the solution according to the room temperature; so, when it is cool, the concentration must be higher than when it is warmer. The suitable temperature is 15°–18°C; if it is too warm, that is higher than about 20°C, the nervous tissue is coloured too deeply or too faintly, and a good preparation can not be made.

4) Injection method. — The staining solution usually employed for injection is of the following composition; standard solution 1 vol. physiological solution 4–7 vol. This diluted solution is injected into the body cavity of the worm by means of an injection apparatus with a needle of 1/5 mm. in thickness. The amount of solution to be used depends, of course, upon the size of the worm. For instance, for the newly born worm, an amount less than 1/5 cc. may be sufficient, while for the worm 10 weeks old even more than 1 cc. will be necessary. Here again the duration of staining is a difficult problem, so the adequate point must be determined by repeated trials. For newly born earthworms, the time needed for that purpose depends upon the temperature and the use of hydrogen peroxide. When the room temperature is warm, being higher than about 20°C, from fifteen to sixty minutes will be sufficient in the vapour of hydrogen peroxide. Usually after ten or twenty minutes, the superficial nerves begin to stain, but the dye does not reach all of the neurons but, if the hydrogen peroxide is used, their staining occurs very rapidly. And moreover, it is especially necessary to regulate the concentration of the solution, so as to make the body tinged with light blue by the injection. If the body wall of the worm becomes suitably light blue about a minute after the injection, we can anticipate success for a good preparation. If, however, the specimen is coloured too deeply or too faintly a good result can not be obtained in staining the nervous element.

During the staining the worm must be frequently examined under the microscope. If the concentration of the rongalit white solution and the air temperature are adequate, the nerves are stained more easily than other tissues within a short time; in addition, the nerves have the characteristic of keeping the blue colour for a longer time than other tissues. In cases of young embryos, I have applied at first a thicker solution for the staining, and after all the tissues were equally stained, the material was transferred to the ordinary diluted solution and then I waited until all the other tissues except the nervous elements faded completely. If during this procedure the air temperature is high we put the materials in the vapour of hydrogen peroxide and then the staining will be successful. Generally, it is considered that staining with methylene blue does not go well in summer, but we have usually obtained good preparations using hydrogen peroxide even in 30°C of room temperature. When working in summer, I have at first done injection and afterwards applied the submersion method; it was not desirable in higher temperature to use simply the injection.   •

5) Fixation. — When the staining is at its best, we must immerse the material in water or physiological solution to wash out thoroughly the residue of the rongalit white. For the fixation I employed exclusively the saturated aqueous solution of ammonium molybdate. If the worm is large, before pouring the fixing fluid on it, we must cut off the desired parts from the body and put them into the fixation bath. But, if it is small, this procedure is unnecessary. In the fixing fluid cotton must be laid at the bottom, and the materials must be placed in a situation proper for the observation. I used also ammonium picrate as the fixing agent, but only in some particular cases. In the fixing solution the materials were generally left from one to three hours, but sometimes in cases of small worms only fifteen minutes was enough. On the other hand, the duration of fixation must not be too long, for the stained nerve will lose its colour gradually by being for a long time in the fluid, such as ten to twenty hours. Then in order to wash out the ammonium molybdate they should remain in the distilled water for various lengths of time according to their size. The distilled water must be frequently changed. If the washing is not sufficiently done, the crystals of ammonium molybdate will appear and decrease the clearness of the preparation. After washing in the distilled water the materials were immediately put into absolute alcohol; if we immerse it in alcohol of lower concentration, even for a very short time, the stained objects will gradually lose their

colour. After being dehydrated in alcohol, they are made transparent in xylol and then mounted with balsam in the usual manner. Mostly the materials were observed without sectioning, but sometimes they were cut by free hand in order to study more in details the structure of the nervous elements.

I also made some experiments to obtain a better fixation of the tissues. Of the various reagents, with which I have experimented, only formol does not seem to damage the staining property of the nervous tissue. But, it gives a precipitate with ammonium molybdate solution. As the mixture of ammonium molybdate and formalin remains clear for some minutes, I profitted this property in the following way; to the solution of ammonium molybdate formalin was added (1 cc. of formalin to 100 cc. of the solution) and the materials were immediately put into it. Within 10 to 20 minutes this fluid becomes turbid and has to be replaced by a freshly prepared mixture. The change of solution must be repeated, and afterward the objects are submerged in the solution without formol. This process is rather difficult, but it is worth while trying when the simple solution of ammonium molybdate appears quite useless. Some good preparations have been obtained by this method, but generally I have used the more simple one, which was mentioned earlier.

B) For counting of the nerve cells and fibers.

For this purpose I have used the same method as in my previous experiments. The nerves together with the body wall of every period of development of the earthworm were fixed in LAVDOWSKY's fluid. The materials thus treated were embedded in paraffine, cut transversely or longitudinally 8 or 10 micra in thickness and stained with HEIDENHAIN's haematoxylin. To ascertain the number of the ganglion cells, I have counted the number of their nucleoli, while for the nerve fibers, only the motor fibers were treated. The sizes of the nerve cells and other cells were measured in most cases in the section passing through the nucleus. As for muscle fibers the isolation method was employed with 32% nitric acid and 20% potassium hydrochloride.

Though it is desirable to observe as great a number of specimens as possible, in practice I have treated only from five to twenty worms for each growth phase, owing to the difficulty of preparing the specimens.

IV. OBSERVATION OF THE NERVOUS SYSTEM BY METHYLENE BLUE METHOD

GENERAL REMARKS

The methylene blue preparations are very excellent for examining the precise form of neurons, though they do not reveal cytological details. By this method, the cytoplasma shows various features during the staining process. At the beginning of staining numerous pale blue small granulations appear in the cell-body. After some minutes the granulations become completely colourless; so we can not then discern the cell-bodies themselves. But, after two or three hours they begin again to take colour uniformly and gradually the colour deepens. It is probable that the former reaction occurs in the living cells, while the diffuse staining takes place in the elements which are dying. The nuclei are variable in their character; some of them are faintly coloured, while the others are stained deeply. In general, they seem to show the reverse relation of stainability against the cytoplasma, i. e. the nucleus assumes deep coloration in the cell, whose cell-body is lightly stained, and vice versa. But, after the cytoplasm has taken a deep blue colour the nucleus can no longer be distinguished inside the cell. On the other hand, all other tissues except the nerve cells are stained very little by this methylene blue method. If the staining be successful, we can trace the whole course of nerve fibers to the terminal point, where they end with a bud-like swelling. Generally speaking, as the results of the vital staining method by methylene blue are very capricious, as well known, I had to observe many preparations in the same stage. Since the greatest fault of this staining agent is its difficulty of penetration, as already mentioned by KRAWANY, I have employed practically only young worms of various growth periods. The most convenient materials are the embryos, which are still in their cocoons measuring 2 cm. in length, as they have no pigment and give good results for observation, and the main neurons were stained in this case up to the connection between them. Furthermore, in young specimens the anatomy of the nervous system can be easily studied by this method, for the peripheral nerve trunks leaving the central nervous system are then plainly tracable to their end organs. The results of my observation will be noted under the following subjects;

1) The ventral nerve cord in the typical segment.
2) The suboesophageal ganglion.

3) The circumoesophageal connective.
4). The cerebral ganglion and enteric nerve.
5) The intermuscular nerve cells.
6) The sensory nerve cells in the epidermis.

1) THE VENTRAL NERVE CORD

In the study of the nervous system cf the earthworm the ventral cord has been studied histologically by a great number of earlier workers· (CLARKE '56, FRIEDLÄNDER '88, HALLER '89, CERFONTAINE '92, RETZIUS '92, KRAWANY '05, HALLER '10, etc.). Of these reports only that of CERFONTAINE was not accessible to me. The accounts of these scientists, however, do not at all agree with each other; some of the differences are due probably to the unsimilarity of their methods. In the present work, the paths of neurons in the ventral cord, especially the manner of connection between nerve fibers was observed by the vital staining of the neurons in each growth period. The ventral cord is generally divided into three parts; the first part is the ganglionic cell layer, where numerous nerve cells are gathered in the ventral and lateral regions of the cord; the second is the neuropile where a great deal of nerve fibers pass through from the suboesophageal ganglion to the tail end ganglion; and the third part is the region of the giant fibers, which run in the dorsal side of the cord along the neuropile. In the ganglionic cell layer there are various sorts of nerve cells, tripolar and multipolar ones, as described by the earlier investigators. Rarely I have found seemingly apolar cells, such as to be seen in Tafel I, Fig. 2 by RETZIUS, while the majority of the cells in the ventral cord are unipolar in shape, and the nerve cells with two or more processes are also scanty in numbers.

Otherwise, I have distinguished two groups in neurons of the ventral cord; one of them is motor nerve cells giving off their processes to the periphery through segmental nerve trunks, while the other is associative nerve cells which are called "Binnenzellen" by KRAWANY ('05), and "Interganglionic nerve cells" by SMALLWOOD ('27); some of the latter are very small in size, and have short processes (Fig. 10 and Tafel XV, Fig. 2).

1. The motor cells. — The motor nerve cells send their long axons to the periphery by way of the segmental nerve trunks to supply innervation to a large quantity of muscle fibers, and they run partly across to the other side of the ganglion to get into one of the nerve trunks or

else leave the ventral cord without crossing through a nerve trunk on the same side as the cell-body (Fig. 3). In general, the motor ganglion cells, which give off their axons to muscle fibers, are larger in size than other ganglion cells, the largest ones measuring about 60 micra in diameter. They present in the methylene blue preparations various appearances; very often their nucleus shows a deep blue coloration, while the cytoplasm assumes a light one, and the nucleolus is coloured more deeply than the nucleus itself. A careful inspection of the axons and dendritic branches shows that there are two general relations; One, the formation of numerous fine collateral branches in the neuropile of ganglion to get contact with the giant fiber, as mentioned by SMALLWOOD ('27), and the other, after leaving the ganglion the long axons innervate the muscle fibers giving off many branches in their course. According to my observation the number of collateral branches issuing from the motor nerve fibers is much greater than one expects from the reports of the
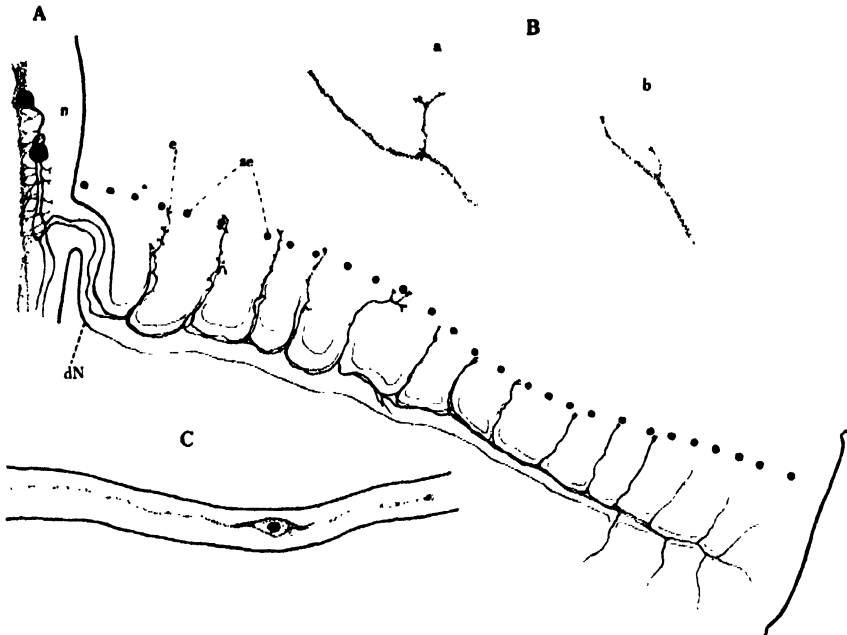
Fig. 2. A) Two motor neurons giving off many branches to the muscles of the setae in the newly born worm. e = ending, n = motor neurons, se = setae, dN = double nerve; ×100. B) The nerve endings in the newly born worm. a) dendritic nerve ending in the proximal portion of the axon, b) bud-like nerve ending in the distal portion of the axon; ×400. C) The process of an intermuscular nerve cell in the single nerve of a 15 mm long embryo. ×400.

earlier workers. Plate XV, Fig. 1, and Textfig. 2 indicate two motor ganglion cells in the embryo of 20 mm. in body length which serve for the movement of setae; they give off numerous collateral branches which come in contact with the giant fibers in the ganglion, and many other branches to every seta.

From the observation of about fifty preparations, the nerves which were more or less easily tracable are represented in Figs. 3–10; and in practice the nerves observed with certainty are drawn with continuous lines, while the others which could not be fully traced are indicated by dotted lines.

As SMALLWOOD has described them, the motor nerve fibers in the tissues are here and there thickened and granular; therefore, their size is not uniform throughout their whole length. A careful inspection of the structure of the fibers indicates (Plate XV, Fig. 1) that each has one neurofibril which arises from near the nucleus, and is surrounded by granular substances.

Under the high power of the microscope I observed various stages of development of nerve ends in muscle fibers of embryos and young worms; the end of the nerve presents itself as bud-like, when it is not fully developed, while the fully grown nerve end assumes the dendritic manner, which is the nature of the almost all nerve ends in the fully grown worms. The gradual steps of growth of the nerve ends can be well studied in a newly born or young worm. As illustrated in Fig. 2, B, in one neuron the proximal nerve endings have already become dendritic in shape, while the distal ones stay yet in bud-like form. These facts indicate, according to my opinion, the gradual development of the nerve end. Recently, H. McCONNELL ('33) studied by the vital staining with methylene blue the development of ganglion cells lodged in the nerve net in buds of *Hydra*, and reported facts about the growing nerve processes similar to those observed by me in the earthworm. This similarity seems to be interesting in view of the phylogenetical relation.

2. *The motor neurons sending off their axons to the single nerve.* — Fig. 3, A indicates the motor neurons, whose processes leave the ventral cord through the single nerve in the XXXth segment of the almost full-grown worm (10 weeks after hatching), and in Fig. 3, C these cells in the embryo (which is 20 mm long) are shown. In most of these, the axons are heterolateral, but in some they take the homolateral course. According to my own results, there are 9 different types of neurons, which send off their processes into the single nerves. Recently, PROSSER

('35) constructed diagrams of the paths of neurons, using the descriptions
by earlier workers (CERFONTAINE '90, RETZIUS '92, KRAWANY '05, and
HALLER '10), in which the motor and associative neurons are schematically
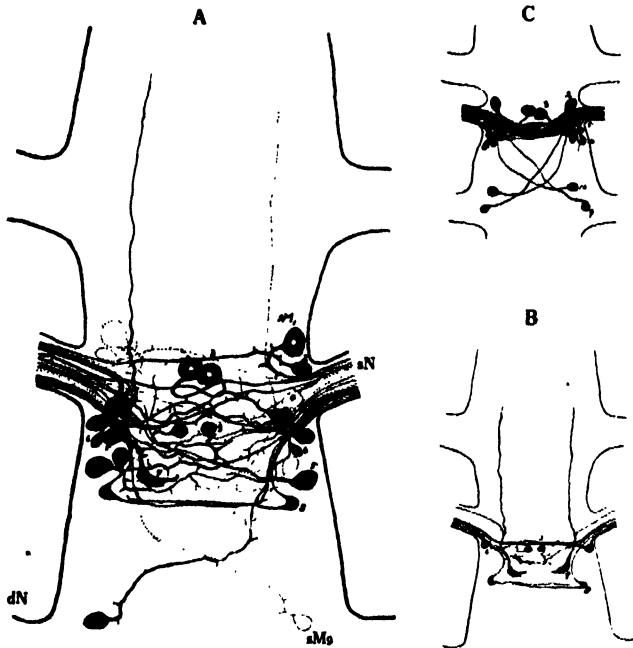presented. I also made abstracts in Fig. 4 of the forms of neurons

Fig. 3. The motor neurons sending their axons into the single nerve in the
typical segment; ×9. A) All the neurons stained in the 10 weeks old worm. B)
Conspicuous neurons in a 10 weeks old worm. C) Neurons in a 10 mm long
embryo. sM₁—sM₁₀ = motor neurons.

sending their processes into the single nerves from the reports of the
authors mentioned above (from the original works of RETZIUS, KRAWANY,
and HALLER, and, as to CERFONTAINE's opinion, from the book of FORTUYN,
1920). From the comparison between Figs. 3 and 4, it may be recognized
that there are several types of neurons in addition to the types which
were figured by the four investigators (Fig. 4). First I wish to mention
here that the types of neurons described by the earlier workers, summarized
in Fig. 4, are seemingly the same as those discovered in my observation.
The homolateral type, " sM₄," in Fig. 3, may be equal to " M₂ " in
HALLER's figure. On the other hand, some of the heterolateral types,
which are situated in the lateral region of the ganglion, and named " sM₂"
and " sM₃" in Fig. 3 seems to be the same type as " m₃" (Krawany),

"b" (CERFONTAINE) and "d", "k" (RETZIUS) in Fig. 4. Furthermore the other cell type, "sM_3", which is located in the mid-ventral side, seems to correspond to "d_m" (RETZIUS) in Fig. 4. Next I shall describe here a few types of neurons (Fig. 3, "sM_6", "sM_7", "sM_8", and "sM_9"), which are not found in the observations of the above mentioned workers. One
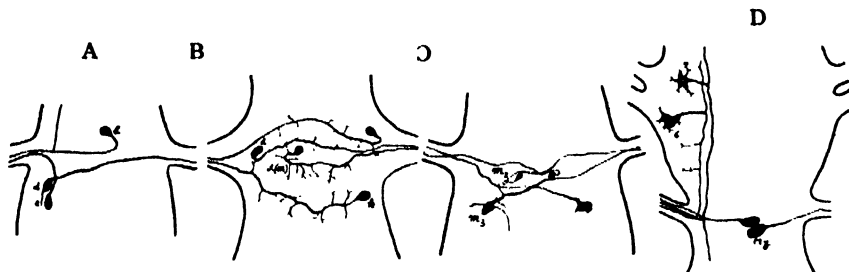
Fig. 4. Diagrams upon the types of neurons belonging to the single nerve trunks.
A) Neurons in *Lumbricus* by CERFONTAINE;   B) Neurons in *Lumbricus* by RETZIUS;
C) Neurons in *Eisenia* by KRAWANY;   D) Neurons in *Lumbricus* by HALLER.

of these types gives off two processes, one of which runs through the neuropile of the adjacent ganglion of the ventral cord and the other goes out into the single nerve trunk (Fig. 3, B, "sM_7"), while the other nerve cells (Fig. 3, C, "sM_3") are localized in a region far from the base of the single nerve through which their axons go out.

On the other hand, in the young embryo (Fig. 3, C) motor neurons present nearly the same features as in the 10 weeks old worm, except one type of cells ("sM_{10}") which perhaps did not get stained in the latter specimen. As to the neurons of the emhryos, it is especially remarkable that the "sM_2", "sM_3" and "sM_5" could be recognized in so early stage as in embryos (which are 10 mm long). They consisted of about fifty segments, whose embryonal setae are arranged in two rows, and the nephridia also in the ventral sides are arranged in two rows. As the neurons, "sM_2" and "sM_3", were found also in *Eisenia* and *Lumbricus* they may be significant in the phylogenetical relation. Finally, the number of stained motor neurons in the methylene blue preparation is much smaller than that of the motor nerve fibers coloured by the haematoxylin method. As Tables II and III show, the motor nerve fibers contained in the single nerve are more numerous than the stained nerve cells in the corresponding ganglion, the ratio, for instance, between them being 20 : 15 (fibers : nerve cells) in 10 weeks old worm, although some of the neurons were not stained in this preparation. And moreover, besides these coarse

nerve fibers, an amount of small motor fibers passes through the single nerve trunk. I could not, however, ascertain their connection with the nerve cells in the ganglion. On the other hand, in young embryos, such a discrepancy as mentioned above, does not exist between nerve fibers and cells, the number of the former coming in this case very near to that of the latter. The above mentioned facts, according to my opinion, depend upon the more easily stainable character of nerve cells in embryos, in which probably all of the neurons take the dye.

3. *The motor neurons sending off their axons into the double nerve trunk.* — In *Pheretima*, the second and third of the segmental nerve trunks are called double nerve, as they are fused at the base to a common root; they are, in other words, the anterior and posterior trunks of the double nerve.

    i) *The neurons belonging to the 2nd nerve trunk (anterior trunk of the double nerve).*

According to four workers mentioned above, there are various motor neurons sending their axons into the second nerve trunk; their views upon this question are summarised in my Fig. 7. My own observation
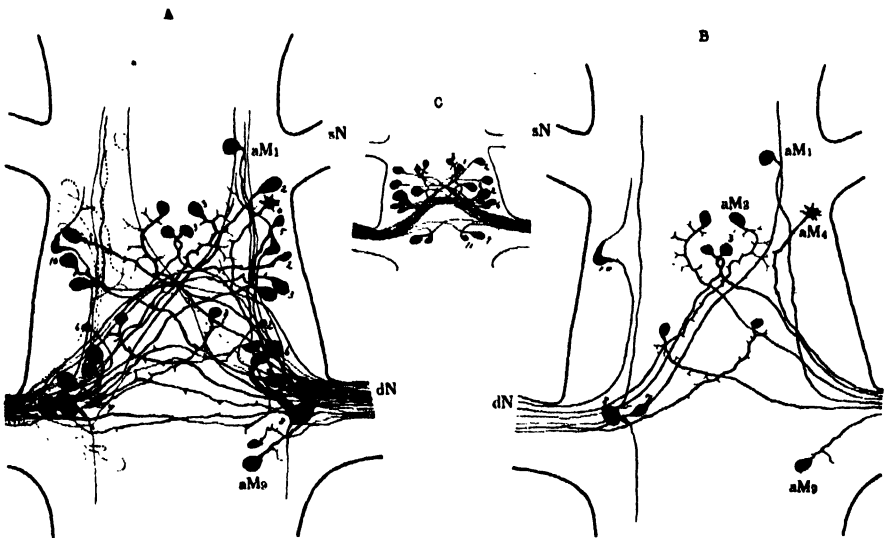
Fig. 5. The motor neurons sending their axons to the anterior trunk of the double nerve in the typical segment; ×90. A) The motor neurons in the 10 weeks old worm; B) Principal neurons in the same worm; C) The motor neurons in the 10 mm long embryo. aM_1-aM_{11}=motor neurons, sN=single nerves, dN=double nerves.

of more numerous neurons are represented in Fig. 5.   When we compare Figs. 5 and 7, there are some neurons in the two figures which bear resemblance to each other; one of the homolateral neurons, " aM₁," of Fig. 5 beginning at the base of the single nerve seems to be the same type as " m₁" in KRAWANY's figure (Fig. 7), and the others, " aM₄", and " aM₅," which are located in the lateral regions correspond probably to " h " by RETZIUS, and " m₄" by KRAWANY (Fig. 7).   Fig. 5 shows also a multipolar cell " aM₄".   Lastly, " aM₆" and " aM₉" may be similar to " i " by CERFONTAINE.   On the other hand, as to the heterolateral neurons, " aM₂" in the lateral region of the ganglion seems to be the same as " g " by RETZIUS, and also the same as " c " by CERFONTAINE, while " aM₃" which gives off numerous branches corresponds probably to " m₅" by KRAWANY (Fig. 7), and to " e " by RETZIUS.   But I could not find any neurons, which were similar to " 1 " and " 6 " in HALLER's figure (Fig. 7).   Besides those illustrated by earlier workers there are, to my observation, still other different types of neurons; namely the cell-types, " aM₇", " aM₈", and " aM₁₀" as shown in Fig. 5, B.   Even in the embryo, almost
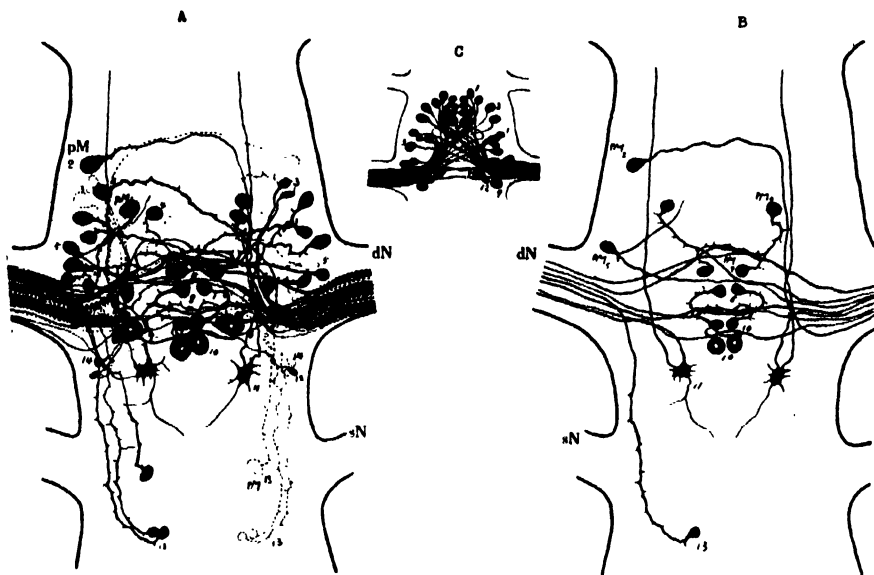
Fig. 6. The motor neurons belonging to the posterior trunk of the double nerve in the typical segment; ×90. A) All the motor neurons stained in the 10 weeks old worm. B) Principal neurons in the 10 weeks old worm. C) The motor neurons in the 10 mm long embryo. pM₁–pM₁₄=motor neurons, sN=single nerves, dN=double nerves.

all the neurons to be seen in the full-grown worm seem to be present, though they can not all be seen in the preparations. It may be significant from the viewpoint of phylogeny that " aM_1", " aM_2", " aM_3" and " aM_4" which are represented in *Eisenia* or *Lumbricus* are also present in the embryo of *Pheretima*. Furthermore, I ascertained that the number of nerve fibers leaving the ventral cord through the second nerve trunks of both sides exceed that of the neurons seen by the methylene blue method in the approximate ratio $2:1$. This ratio holds true in the full-grown worm of more than 10 weeks after hatching, but approaches to $1:1$ in the embryos.

ii) *The neurons belonging to the 3rd nerve trunk (posterior trunk of the double nerve).*

As well known, the motor cells which send their fibers into the posterior trunk of the double nerve are very abundant. In this trunk are contained conspicuous coarse motor fibers, such as seen in Fig. 6; some of them can be traced to the muscle of the setae giving off collateral branches to it (Fig. 2). At first, among the homolateral neurons, one type " a " by RETZIUS is closely similar to my " pM_3", which was usually stained well and very conspicuous even in an earlier embryo (10 mm long) by its clear appearance. On the other hand, " m_6" described by KRAWANY probably corresponds to my " pM_1". My further attention was directed to " pM_{13}" one of the homolateral neurons, which is located in the caudally succeeding ganglia and they have not been mentioned by the previous workers. In addition to the neurons mentioned above, smaller neurons are localized in the base of the double nerve trunk, as they are indicated in Fig. 6, " pM_{11}", and " pM_9". On the other hand, there are also numerous types in the heterolateral neurons; " m_8" by KRAWANY and " 4 " by HALLER may be similar to my " pM_2", which is already present in the embryo, while " e " by RETZIUS seems to correspond to my " pM_3", and " pM_{10}", though one of them (" pM_3") was not seen in the embryo. Moreover, the so-called median large ganglion cells by HALLER " m " bear a resemblance to my " pM_{10}" as to the position, but the former are bipolar, giving off the axons into the second and third nerve trunk, while the latter is unipolar, and its axon goes into the third nerve trunk. In Fig. 6, C, especially, conspicuous types of the neurons belonging to this trunk are represented as " pM_2", " pM_4", " pM_5", " pM_7", " pM_{10}" and " pM_{11}". Among these neurons, the multipolar one is named as " pM_{11}", but I could not find the cells corresponding to " 2 " and " 3 " by HALLER. Upon the numerical relation between the number of efferent fibers and

of the neurons belonging to the third trunks, the large excess of about two of the former against one of the latter is seen in the full-grown worm,
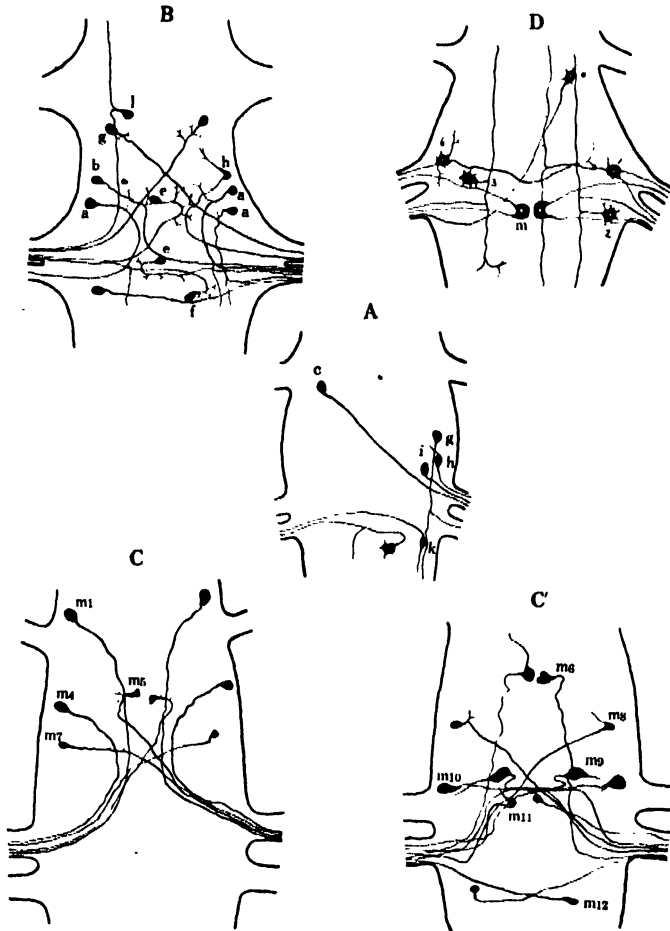

Fig. 7. Diagrams upon the types of the motor neurons belonging to the double nerve trunks. A) Neurons in *Lumbricus* by CERFONTAINE; B) Neurons in *Lumbricus* by RETZIUS; C′, C) Neurons in *Eisenia* by KRAWANY; D) Neurons in *Lumbricus* by HALLER.

while in the embryo most of the nerve cells are seen in continuance to the efferent fibers owing to their good staining capacity.

4. *Interganglionic nerve cells.* — During my previous experiments I found that there are numerous ganglion cells, whose axons do not enter into the peripheral nerve trunks; they are called 'Binnenzellen' (KRAWANY),

commissural nerve cells (PROSSER), or interganglionic nerve cells (SMALL-WOOD). In these cells, which occupy more than half the number of those appearing in the various types, I shall distinguish three kinds; the first one is gross cells described by KRAWANY, RETZIUS, etc. as motor cells, the second one is very small cells (Fig. 10 and Plate XV, Fig. 2) having a
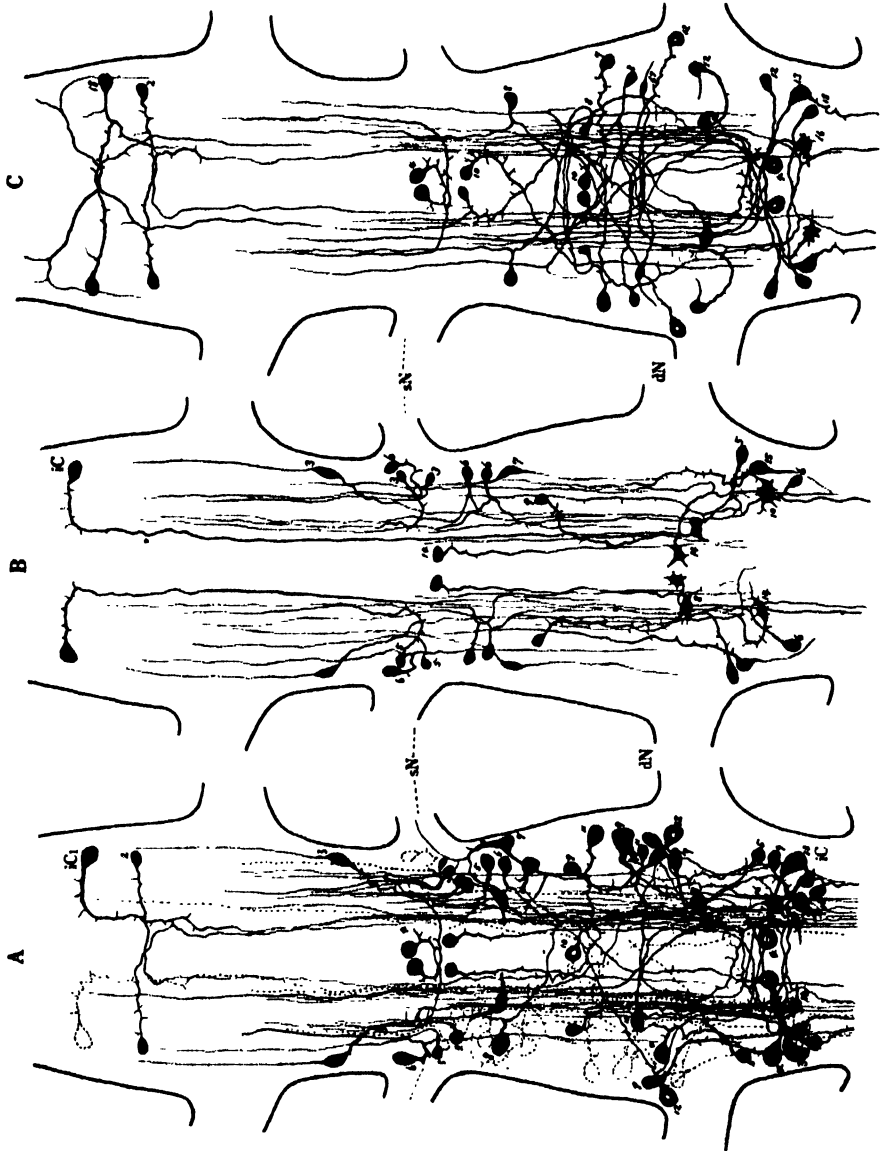
Fig. 8. The interganglionic (commissural) nerve cells in the typical segment; ×90. A) All the neurons stained in the 10 weeks old worm; B) The homolateral neurons; C) The heterolateral neurons; D) All the neurons stained in the 10 mm long embryo. iC_1-iC_{18}=interganglionic nerve cells, dN=double nerve trunks, sN=single nerve trunks.

diameter of 7-10 mm, and the third one is multipolar cells illustrated by RETZIUS.

The gross cell type is represented in Fig. 8; the axons of some of these cells pass over to two or three adjacent ganglia, either crossing or not, while the others terminate in the neuropile of the same or opposite side in each ganglion. Most of these cells are, to my observation, unipolar and each of their single processes gives off numerous collaterals during its course; the rest is bi- or multipolar, and the great many collaterals issue from their processes. In Fig. 9 the views of four previous investigators upon the cells in question are summarized, and in Fig. 8, A, B, C, D, my own results upon the problem are drawn semi-schematically. In the first place, as to the homolateral neurons (Fig. 8, B), " n " by RETZIUS, "8" by HALLER and "f" by CERFONTAINE, seem to be correspondent to " iC_6 " in my Fig. 8, while " S_7 ", " S_8 ", " S_{10} ", " S_{11} " and " S_{14} " by KRAWANY resemble my " iC_3 ", " iC_9 ", " iC_7 ", " iC_{11} " and " iC_5 " respectively. Besides, there are found various other types of the homolateral neurons, namely " iC_1 " in my illustration, whose cell-body lies in the far region of the adjacent rostral or caudal ganglion, and whose long axon is distributed to three succeeding ganglia, and also " iC_{14} " and " iC_{15} ", which assume curious features; they are almost all multipolar. On the other hand, among the heterolateral ones (Fig. 8, C), " n " by RETZIUS, " 5 " by HALLER which are located in the lateral part of the ganglion may be similar to my " iC_{13} ", while " k " by RETZIUS and " S_1 " " S_5 " by KRAWANY situated in the mid-ventral side of the ganglion seem to correspond to my " iC_4 " and " iC_{10} ", though their axons extended in my specimens both to the anterior and posterior directions. Moreover there are many types of

heterolateral neurons, which were not mentioned by previous workers. "D" in my Fig. 8 shows embryonal commissural cells; not all but the main parts of them observed by me are represented in the drawings.
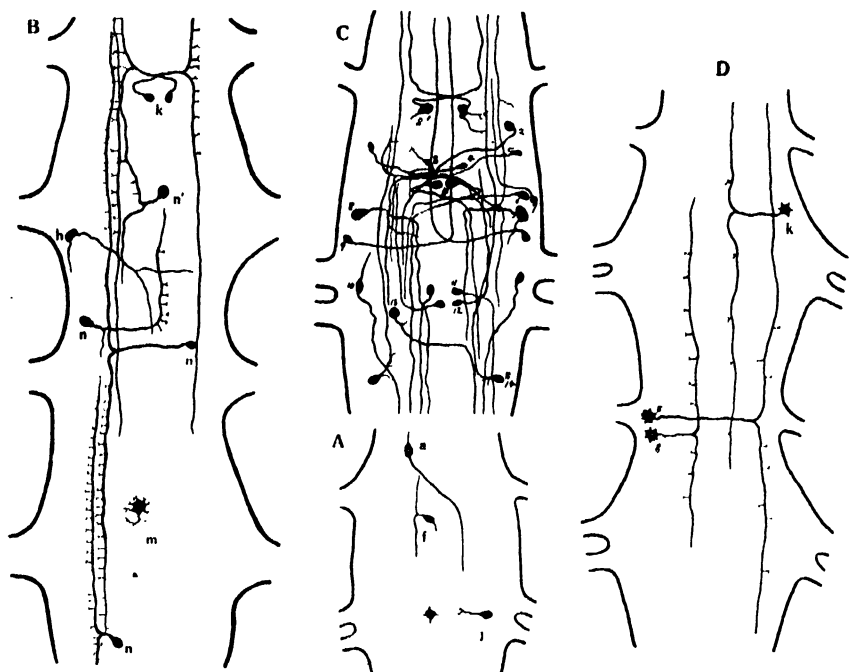

Fig. 9. Diagrams upon the types of the interganglionic cells reported by previous authors. A) Neurons in *Lumbricus* by CERFONTAINE; B) Neurons in *Lumbricus* by RETZIUS; C) Neurons in *Eisenia* by KRAWANY; D) Neurons in *Lumbricus* by HALLER.

The second type is unipolar small cells each possessing a short process, and their number is about half of that of all the neurons in the typical ganglion. They are especially in the complicated ganglia of the head region, and in the suboesophageal ganglion. Furthermore these cells can be distinguished from the gross ganglion cells (the first type) by their processes and nuclei. The distribution of these small commissural cells is shown in Fig. 10, A and Plate XV, Fig. 2. So far as I am aware, no account has been published concerning the commissural neurons, with the exception of that by CERFONTAINE on the cells named "j" which seem to be similar to the commissural neurons. I have not been able to ascertain the nature of these neurons but some of their groups send the
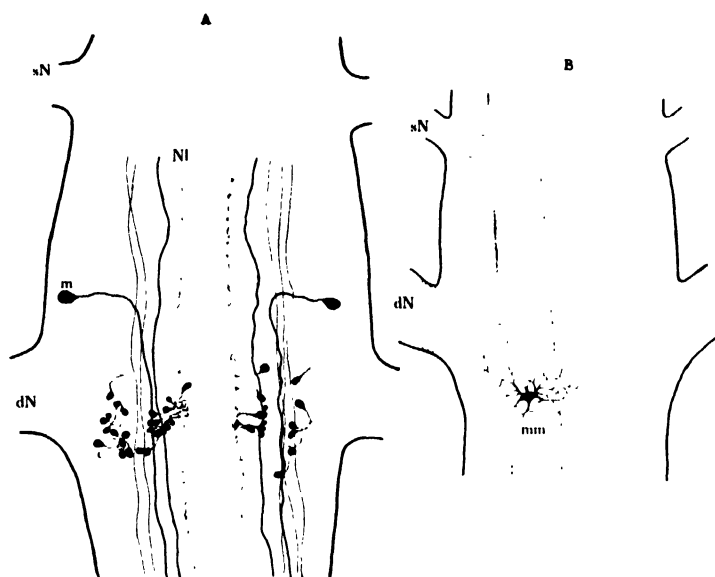
Fig. 10. A) The small commissural neurons in the typical ganglion; ×90. B) The multipolar ganglion cells, c — commissural neurons, dN — double nerve trunks, m — motor neurons, mm — multipolar cells, Nl — neuropile, sN — single nerve trunks.

process to a definite part of the neuropile, while the others are distributed at random outside of the latter. Also they resemble the small cells in the cerebral ganglion, which will be described in detail below.

In this respect it may be worth mentioning, that the number of nerve fibers running through the neuropile of the internode examined in the haematoxylin preparation, even in the embryo is much larger than that of neurons, which extend their processes to the neuropile. This fact is more remarkable, as there are also abundant small nerve cells, which have only short processes, in the ganglion. Perhaps, this fact may be caused by the summation of fibers, which pass over several adjacent ganglia, making the processes of bipolar, tripolar and multipolar cells. This fact may also depend upon the circumstance that the fibers stained by methylene blue are fewer in number than those to be seen in haematoxylin preparation.

The third type represents gross multipolar ganglion cells which lie in the middle portion along the median giant fiber (Fig. 10, B). Perhaps they are not identical with the apolar cells described by RETZIUS.

5. *Sensory nerve fibers in the ventral cord.* From the periphery, numerous sensory fibers come into the ventral cord through three segmental nerve trunks. On entering a ganglion, the sensory nerve fibers take

various courses which are shown in Fig. 12, A and B. As seen in Fig.
11, these sensory fibers form three bundles passing through the ventral
cord, as mentioned by RETZIUS. The majority of them are divided in
two branches of T- or Y-shape as noted by RETZIUS and LENHOSSÉK; as
soon as the sensory fibers enter the ventral cord and divide into branches
extending to the anterior and posterior directions, they give off numerous
collaterals. In the dorsal view of the ventral cord, these collaterals seem
to end after bifurcating in two small branches in the definite lines of both
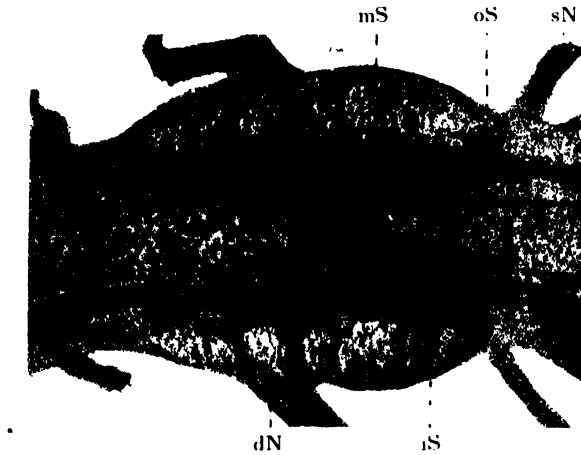
Fig. 11. The sensory bundles in the neuropile of the ventral
cord (newly born worm); × 100. dN -double nerves, iS—inner bundle,
mS = middle bundle, oS—outer bundle, sN -single nerves

sides, which pass along the lateral and ventral giant fibers; Fig. 12, A
shows these relations. This fact has been already confirmed in *Eisenia*
by SMALLWOOD. Moreover we see other branches (" bf ") in the surface
view and cross-section which cross over the middle line and terminate in
the opposite side. This type of the sensory fiber was not discovered by
RETZIUS and LENHOSSÉK, but the endings of the collaterals of this fiber
as described by RETZIUS seem to resemble those of my own observation,
while his description of the manner of the bifurcation of this fiber in the
ventral cord exactly resembles those in my specimens as shown in Fig. 12.
On the other hand, in the ventral ganglion also the processes of the
motor ganglion cells give off numerous collaterals, which terminate at the
same level where the endings of the collaterals of the sensory fibers are
arranged; therefore in the space between the motor cells and the sensory
fibers there may be a junction of the motor and sensory collaterals. From

my observation of various specimens as shown in Fig. 13, A and B, we may assume that one sensory nerve fiber is not connected to only one motor cell in the ventral cord ; one sensory fiber with many cells and one cell with many sensory fibers seem to be in connection.

6. *Distribution of the segmental nerve trunks.* — All the segments, except in the prostomium and in the last segment, are supplied with three pairs of the segmental nerve trunks which arise from the corresponding ganglion. As seen in Fig. 14, these nerve trunks are distributed in the concerned segment, giving off numerous branches during their courses.

7. *Giant fibers.* — As is well known, the earthworm has three main giant fibers in the dorsal part of the ventral nerve cord, namely a median giant fiber and two lateral giant fibers. In addition in the ventral side of the neuropile of each half a ventral

Fig. 12. The sensory nerve fibers entering the ventral cord through the segmental nerve trunk (typical segment). ×150. A) In the newly born worm. B) In the 15 mm long embryo. bf=sensory nerve fibers crossing over the middle line and terminating in the opposite side, cF=collateral branches of the sensory nerve fibers, dN=double nerve trunks, m=motor neuron, mf=motor nerve fibers, s=sensory nerve fibers.

giant fiber runs through the cord. In all, therefore, five giant fibers run longitudinally in the ventral cord through the entire worm body.

The giant fibers were first described in 1862 by LEYDIG ; he said at that time they were blackened with osmic acid like the myelinated nerve fibers of the vertebrates, and so are remarkably different from the other fibers of the earthworm. Since then numerous investigators have related about the giant fibers. Among them, CERFONTAINE and FRIEDLÄNDER stated that each giant fiber has its cell-body in the posterior part of the ganglion. Also KEYLE asserted that there are two branches from the median and three from each lateral giant fiber in every segment ; he traced some of these branches to large nerve cells in the ventral part of
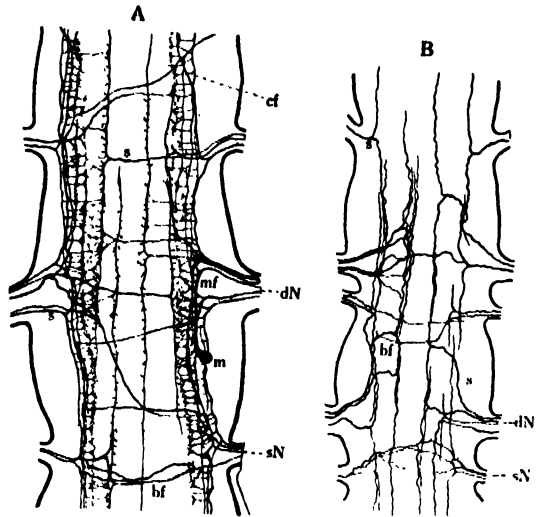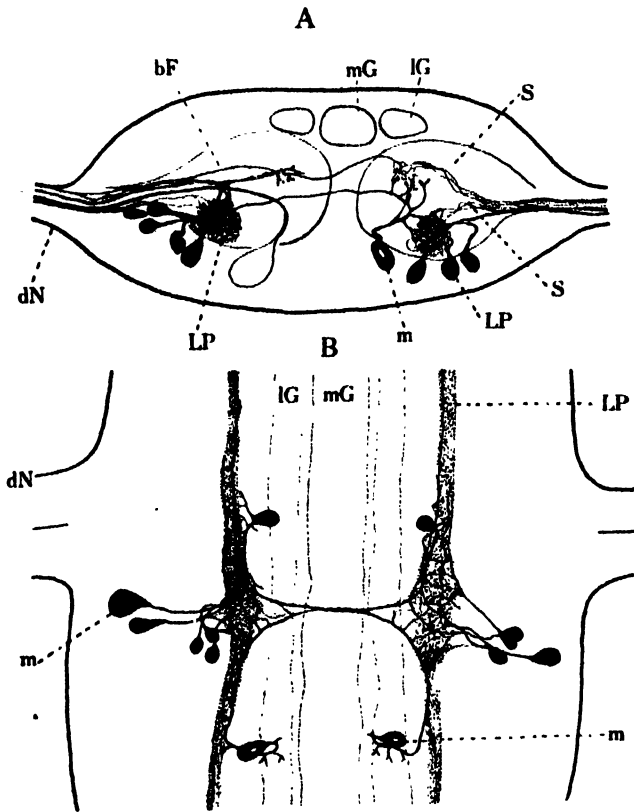
Fig. 13. The relation between the motor neurons and the sensory fibers in the ventral cord. A) The cross section of the ventral cord through the double nerve trunk; ×200. B) The dorsal view of the ventral cord at the same place; ×200. bF=sensory fibers crossing over the middle line and terminating in the opposite side, dN=double nerve trunk, lG=lateral giant fibers, LP=LEYDIG's "Punktsubstanz", m=motor neurons, mG=median giant fiber, S=sensory nerve fiber.

the cord. In 1926 STOUGH made a very intensive study of the giant fibers in *Lumbricus*. He saw connections from the median giant fiber to two pairs of cells and from each lateral giant fiber to one pair of giant cells and to several small ones in every segment. In addition STOUGH stated that each giant fiber consists of compressed axons and that the giant fibers are partitioned by many oblique septa into a great number of cylindrical chambers, which are connected in longitudinal strands at the septa, which may serve as a sort of synapsis, one septum existing in

every segment of the worm body. Afterward SMALLWOOD and HOLMES confirmed STOUGH's remarks and studied further the connection of the ventral giant fibers to ganglion cells or to other nerve fibers in the neuropile.
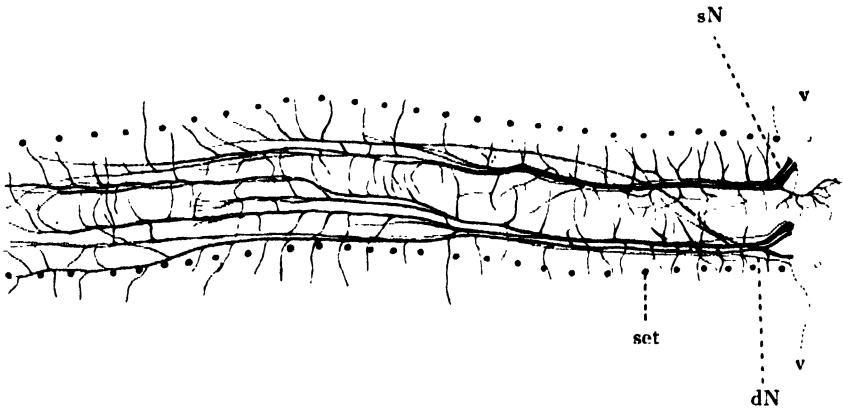

Fig. 14. The distribution of the segmental nerve trunks in the typical segment in the 2 weeks old worm; ×85. dN = double nerve trunk, sN = single nerve trunk, set = setae, v = ventral nerve cord.

In the present work I made also some observations upon the giant fibers by the intravital staining method.

i) The median giant fiber.

The median giant fiber shows clearly the segmental structure by the existence of septa stretching diagonally across the fiber in the posterior part of the ganglion. By the dorsal view, it is readily accepted that several coarse fibers originate from some ganglion cells in the septal region and extend posteriorly passing through the center of the giant fiber to the next septum, at which they terminate with remarkable granular appearances (Fig. 15, A and C). On the other side of the septum the numerous processes of the ganglion cells, which belong to the next segment, terminate with a more profuse branching. Sometimes the median giant fiber is seen probably receiving the processes of a number of special ganglion cells at the septal region, as seen in Fig. 15, E. The neurofibrils are confined in general in the central region of the giant fiber, and, terminating along the diagonal septum, never continue into the next segment. The septa represent, therefore, sharp partitions of the giant fiber.

In addition we see that from the median giant fiber the branches are frequently given off to the neuropile (Fig. 15, A and B), while the lateral giant fibers send their branches into the posterior trunk of the double
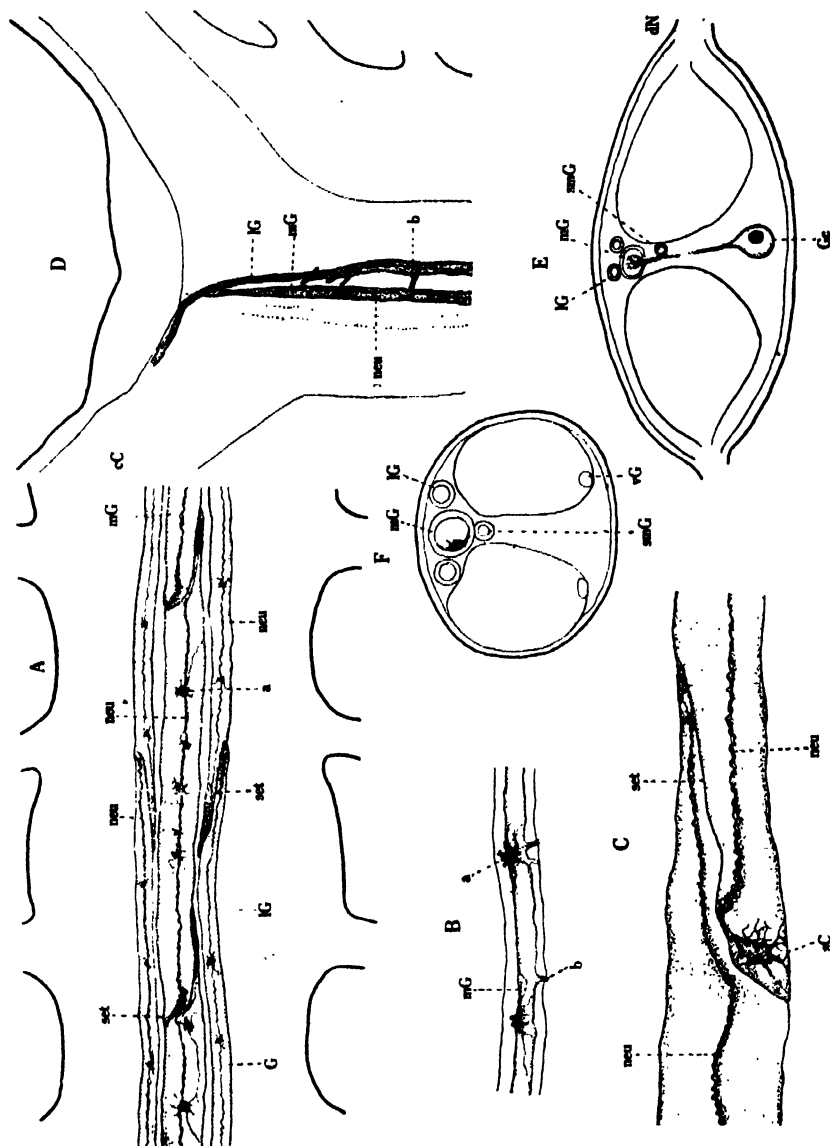
Fig. 15. The giant fibers in the 6 and 10 weeks old worms. A) The surface view of the typical ganglia; ×100. B) The fibrillar substance in the median giant fiber ×150. C) The septum of the median giant fiber ×350. D) The giant fibers in the suboesophageal ganglion ×100. E) The connection of the giant cell with the median giant fiber by the cross section in the 6 weeks old worm; ×150. F) The positions of all giant fibers by the cross section; ×150. a=fibrillar substance, b= branches of the giant fibers, cC=circumoesophageal connective, dN=double nerve trunk, Gc=giant cells, lG=lateral giant fibers, mG=median giant fiber, neu= neurofibrils, set=septum, sC=small ganglion cells, smG=small median giant fiber, vG=ventral giant fibers.

nerve, as they are also shown in the figures by HALLER.

In the suboesophageal ganglion the median giant fiber is recognized as a fine fiber (Fig. 15, D); there it may be said to reach its terminal end, while the lateral giant fibers seem to enter the circumoesophageal connectives of the opposite side crossing over the middle line (Fig. 15, D). Posteriorly the median giant fiber increases in thickness and reaches the maximal diameter in the VIIth segment.

Additionally I wish to mention about the sheath of the giant fibers. These are made for the most part of a certain substance, which bears resemblance to the vertebrate myelin by the staining with osmic acid and by the MALLORY's method. On the outer surface of this myelin-like sheath we see a dense protoplasmic network by the vital staining with methylene blue (Fig. 15, A, B, and G). Here and there the bluish colored cytoplasmic masses are assembled in cell-like manner, and these appearances remind us of the SCHWANN's sheath in the nervous system of the vertebrates. So far as I am aware, such structure has not been reported about the giant fibers of the earthworm.

ii) *The lateral giant fibers.*

The lateral giant fibers exist also segmentally through the entire length of the ventral cord; at the anterior part of the body they are much smaller than the median giant fiber, while the reversal relation of the size is seen near the posterior end. The neurofibrils in the lateral giant fibers originate from certain ganglion cells in the septal region, and contrary to the case of the median one, their fibrils extend anteriorly to the next septum. In general, the sheath of the lateral giant fibers is somewhat thinner than that of the median.

The fibrils, which pass through the lateral giant fibers, seem also to enter these fibers at the septal regions, but the ganglion cells, which are directly connected with the fibrils, were not seen. As shown in Fig. 16 A the branches are given off from the lateral giant fiber into the posterior trunk of the double nerve of the opposite side, issuing on the way many collaterals. The position of the septa, which run diagonally across the lateral giant fibers, is quite different from the position in the median giant fiber, for they are located in the ganglion itself, precisely speaking between the levels of the single and double nerves. Also the direction of the septa of the median giant fiber is contrary to that of the lateral giant fibers. Here again we see on the surface of the myelin-like sheath the dense protoplasmic network, which bears somewhat of a resemblance to the SCHWANN's sheath.
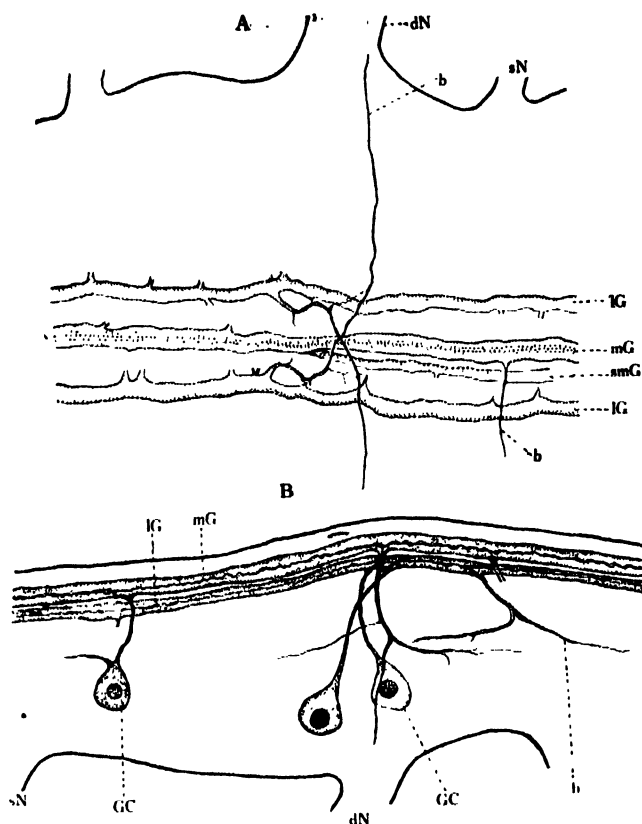
Fig. 16. The median and lateral giant fibers in the 15 mm long embryos; ×400. A) The branching manner of the giant fibers by the dorsal view in the VIIIth segment. B) The connection of the giant cells with the giant fibers in the typical segment. b= branches, dN=double nerves, GC=Giant cells, mG=median giant fiber, lG=lateral giant fibers, smG=small median giant fiber, sN=single nerves.

iii) The smaller median giant fiber.

In *Pheretima communissima* a smaller median giant fiber is found just under the usually so-called median giant fiber; in size the former is almost equal to the lateral giant fiber. Such an accessory median giant fiber has not been reported in *Lumbricus* and *Eisenia*.

As seen in Fig. 16, A, in the 15 mm long embryo the small median giant fiber shows the connection with the main median fiber in the VIIIth segment.

I could not, however, ascertain the connection between the ganglion cells and the small median giant fiber.

iv) The ventral giant fibers.

In the haematoxylin preparation, the ventral giant fibers are found in the ventral side in the neuropile, but by the surface view in the methylene blue preparations they could not be traced clearly enough.

Since in the present experiment the observation of the giant fibers has not been satisfactorily made I wish to study further this problem in the future.

2) SUBOESOPHAGEAL GANGLION.

Although there are a large number of writers, who have reported on the ganglion cells in the ventral cord, the study of the neuronal relations in the suboesophageal and cerebral ganglia has been comparatively neglected. Certain kinds of cells in the suboesophageal ganglion were studied by KRAWANY, but he did not treat then of their connection with the cerebral ganglion. So it is my object at the present time to trace, as much as possible, various types of ganglion cells contained in the suboesophageal ganglion, and to observe in detail also the correlation between them and the cerebral ganglion. The method employed is the vital staining with methylene blue and for the most part the newly born worms were treated since they are the best material for this staining method. The observation was then extended over several stages of growth up to the fully grown worm. Six pairs of the segmental nerve trunks, double and single nerves, issue from the suboesophageal ganglion; in their proximal parts they lie remarkably close together and are distributed distally in a complicated manner to the oral region of the head, as is indicated in Fig. 17. The neurons of the ganglion in question can be classified into several types; and some of these types are characteristic of this ganglion. In addition special cells are met here besides the ganglion cells which are clearly joined to the segmental nerves, and resemble those found in the typical ganglion. In the following, I will describe about neurons in the suboesophageal ganglion comparing my results with those of KRAWANY upon *Eisenia*. My classification of the neurons in question is chiefly based on the direction of processes issued by them.

i) The ganglion cells sending the processes to the cerebral ganglion.

Fig. 18, A shows exclusively the ganglion cells, whose processes extend

from the suboesophageal ganglion into the circumoesophageal connective. Various cells which are characteristic of this ganglion and which could

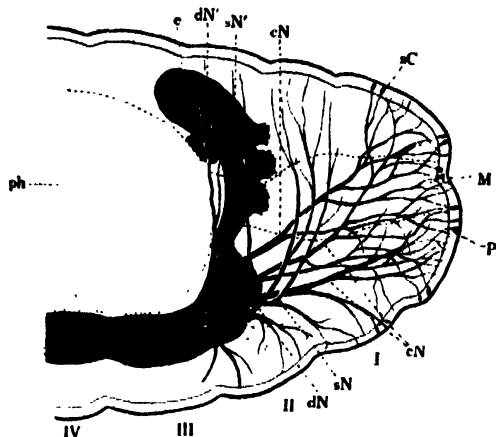be observed by the above mentioned staining, are shown in Fig. 18, A_1-A_6.

In my previous paper ('33) was published the cross section view of the circumoesophageal connective showing nerve fibers of various size studied in haematoxylin preparation. About these fibers, I was at that time of the opinion, that (1) the efferent nerve fibers are partly the processes of cells lodged in the cerebral ganglion, and descend into the suboesophageal ganglion; (2) some of them are given off from the nerve cells in the latter and end in the cerebral ganglion; (3) others originate from the nerve cells in the suboesophageal ganglion, and, passing through the cerebral ganglion, are distributed

Fig. 17. The distribution of the segmental nerve trunks in the right half of the anterior segment of the newly born worm; ×50. cN= nerves from the circumoesophageal connective which pass to segment I and prostomium; e= enteric nerve, dN=double nerve going to segment I, dN′=double nerve going to segment II, p= prostomium, ph=pharynx, sC=sensory cells, M= mouth, sN=single nerve going to segment I, sN′=single nerve going to segment II, I-IV= segments I to IV.

to the periphery by the cerebral nerve trunks of the head region; (4) again others start from the same source, the suboesophageal ganglion and end after passing into the circumoesophageal connective in the thickening of the enteric nerve; and last (5) those, which come from the enteric nerve and reach through the connective to the suboesophageal ganglion. In this chapter those only, whose processes end in the cerebral ganglion, will be mentioned. Fig. 18, A indicates various forms of nerve cells, whose fibers end in the neuropile of the cerebral ganglion. These fibers are gathered in the central portion in the form of a knot in the cerebral ganglion, issuing a great many collaterals. Some fibers ascend from the ventral side so as to end in the dorsal portion of the cerebral ganglion (Fig. 26, A).

Besides, in the suboesophageal ganglion we found another sort of cells, whose axons pass through the circumoesophageal connective and after they entered the cerebral ganglion, divide into many branches, which
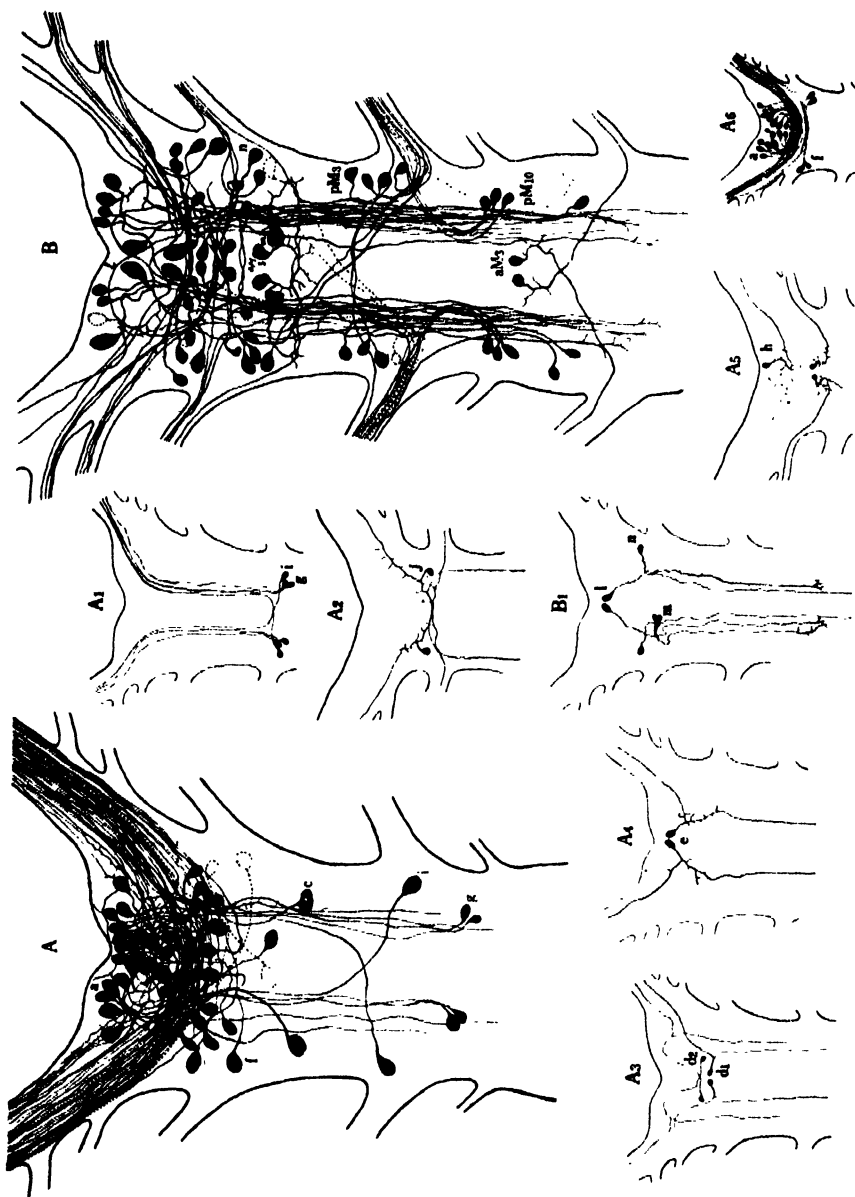
Fig. 18. The neurons in the suboesophageal ganglion, in the newly born worms; ×90. A) The neurons (a–j) sending their axons to the circumoesophageal connectives; and (A₁–A₆) showing the courses of some of them. B) The motor neurons (aM₃, pM₃ and pM₁₀) sending their axons into the segmental nerve trunks, and the interganglionic (l, m, and n) neurons terminating their axons in the neuropile of the ventral cord, and (B₁) showing the courses of some of them.

terminate in ·the neuropile of the cerebral ganglion. These fibers are shown in Fig. 26, A; they were encountered frequently in the suboesophageal ganglion, but have perhaps never been reported by my predecessors.

Moreover, we saw some cells whose processes, after taking the same course to the cerebral ganglion as the axons of the above mentioned cells, leave this ganglion and enter the enteric nerves, where they end with characteristic features after minute ramification. Fig. 27, B indicates these relations.

In the following, I intend to compare my results with the description by KRAWANY of the types of cells which lie in the suboesophageal ganglion and, according to this author, probably send their processes to the cerebral ganglion. KRAWANY says himself that he could not trace them well, for the cells in question were not stained clearly enough.
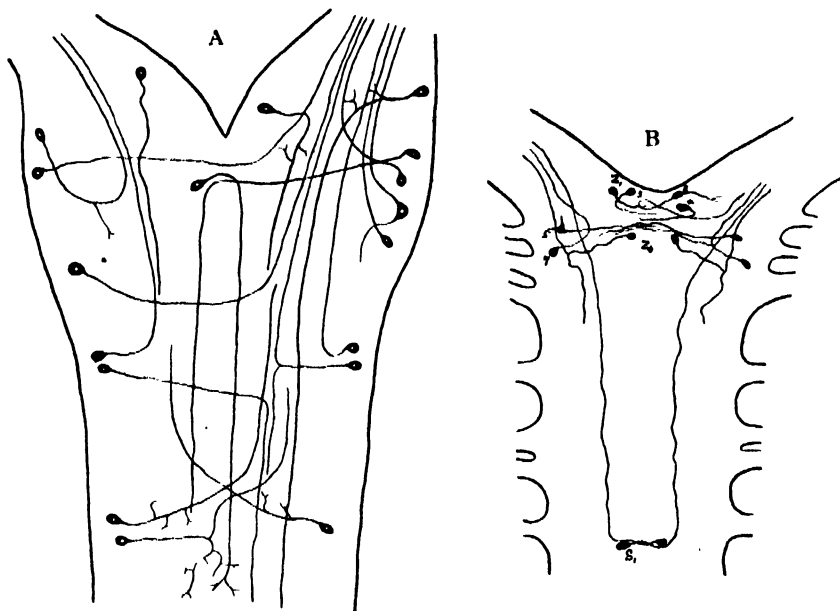

Fig. 19. Diagrams upon the types of the neurons in the suboesophageal ganglion reported by previous authors. A) Neurons in *Nereis* by RETZIUS, B) Neurons in *Eisenia* by KRAWANY.

For the comparison, the neurons in the suboesophageal ganglion ascertained by previous workers (in *Nereis* by RETZIUS, in *Eisenia* by KRAWANY) are summarized in Fig. 19.

The nerve cells, named as " Z_1 " and " Z_3 " in KRAWANY's Textfig. 11,

are unipolar, relatively large ones; they are found abundantly in the most remote anterior portion of the suboesophageal ganglion and their processes enter the circumoesophageal connective of the other side after crossing the middle line. Their existence is surely a characteristic of this ganglion, as KRAWANY also insisted. These nerve cells are shown as " a " in my Fig. 18, A; in reality they are more abundant than we see in the figure. The cells " Z_2 " found by KRAWANY at the frontal end of this ganglion are said to be bipolar, each sending one of their axons in the connective, while the other process ends after branching in the suboesophageal ganglion, but cells of this character could not be identified in my observation.

Several of the next type " Z_1 ", which is unipolar and resemble " Z_1 ", and " Z_3 " in form, but differs from the latter in position, were observed in my materials (" b " in Fig. 18, A_6) in the middle portion of the circumoesophageal ganglion. They are found in pairs and send their axons into the connective of the opposite side. The type " Z_7 " means the unipolar nerve cells, which were seen by KRAWANY in pairs in both sides of this ganglion and send their axons into the circumoesophageal connective of the same side. Probably they are identical with the type " c " in my Fig. 18, A. The number of them is, to my observation, relatively large. Some cells of this type send off the processes, which divide minutely as soon as they enter the cerebral ganglion and go out into the cerebral nerve trunks. Another cell-type, described as " Z_6 " by KRAWANY, and situated in the middle portion of this ganglion, is very characteristic, giving off one process, which is directed lateralwards without crossing the middle line, then bifurcates, one axon entering the circumoesophageal connective, while the other one runs in the posterior direction into the neuropile of the ventral cord. This type resembles the cells " d_1 " and " d_2 " in my Fig. 18, A_3. Also the cells named " e " in my Fig. 18 are somewhat like the above mentioned cell-type " Z_6 " of KRAWANY; the course of the axons is similar, but their position, according to my observation, differs. Besides I observed many small branches sent off near the point of bifurcation of the axons of these cells. The type " Z_5 " in KRAWANY's work is the unipolar nerve cells to be seen in pairs in both halves of this ganglion, their axons entering into the circumoesophageal connective after crossing. They are also tolerably numerous and designated in my Fig. 18, A with " f ". Furthermore, KRAWANY reported a type of cells which connect the ventral cord with the cerebral ganglion under the names " S_{14} " and " S_1 " in his Fig. 11. The cells similar to the type

"S$_{14}$," were also often observed by me and the cells "g" in my Fig. 18, A represent that kind. I did not encounter exactly the same cells as the type "S$_1$," but those named "i" in my Fig. 18, A$_1$ somewhat resemble this type. Their position is different, for "S$_1$" is said to be located in the middle portion, while "i" lies in the lateral side. But both of them send their axons from the more caudal ganglion of the ventral cord into the circumoesophageal connective.

The following types of cells, which I have observed, were not mentioned by KRAWANY. The type "h" in my Fig. 18, A$_3$ represents the nerve cells situated in the middle part of the ganglion giving off numerous branches. Their axons without crossing enter the circumoesophageal connective. The type "j" in Fig. 18, A$_2$ is met with in the lateral portion of the ganglion and sends out one thick process, which divides into three long branches. One of the latter proceeds into the circumoesophageal connective of the same side, another branch leaves the ganglion after crossing the midline through the segmental nerve trunk of the opposite side, while the remaining one runs caudally into the neuropile of the ventral cord.

ii) *Interganglionic nerve cells in the suboesophageal ganglion.*

In the suboesophageal ganglion, there are many interganglionic nerve cells, which send their axons neither into the segmental nerve trunks nor into the circumoesophageal connective, as this is also the case of inter-ganglionic nerve cells in the ventral ganglia. They are partly large, partly small. The processes of the larger cells, the forms of which vary a great deal among each other, reach the neuropile in the caudal portion of the ventral cord. They were called by KRAWANY "Schaltzellen" in the ganglia of the ventral cord. Similar cells also exist, in my opinion, in the suboesophageal ganglion where their existence might be very natural. Almost all of the axons are directed caudally and end either crossed or uncrossed in the neuropile, as Fig. 18, B$_1$ ("l", "m", and "n") indicates. In addition to these large cells, the suboesophageal ganglion possesses many small associative ones, which are drawn in Fig. 10, A, and Plate XV, Fig. 2. These small cells were not noted by KRAWANY.

iii) *Nerve cells, whose processes pass into the segmental nerve trunks.*

As stated before, 6 pairs of segmental nerve trunks are given off from the suboesophageal ganglion and distributed in the prostomium and in the head region (Fig. 18, B). They can be easily observed in the 15 mm long embryos, which have not yet hatched, for the worm bodies do not take the pigment. In them we can see from the outside the nervous

system stained vitally with methylene blue. Concerning the distribution of nerve trunks in the head region, IMAI ('28) published an elaborate schematic figure of *Pheretima megascolidioides*. But his result is somewhat different from my observation in *Pheretima communissima*. In my picture (Fig. 17) the direct continuance of the sensory nerve cells in the epidermis to the nerve trunks and the course of axons starting from the suboesophageal ganglion are taken into special consideration. As the ganglion cells, whose axons are sent into the segmental nerve trunks, are in the most part very similar to the nerve cells in the ganglia of the ventral cord, the details are not rewritten in this chapter, but are shown in Fig. 17.

From the above mentioned we can assume that, the suboesophageal ganglion is functionally an important place of connection with the cerebral ganglion, and secondly it is the ganglion of the ventral cord nearest the head.

3) CIRCUMOESOPHAGEAL CONNECTIVE

In this paragraph I intend to summarize my observations about the various sorts of fibers passing through the circumoesophageal connective. Generally speaking, there are fibers of the upward, or downward conduction; in other words those of the afferent, or efferent nature.

A)

i) Nerve fibers originating in the cerebral ganglion and ending in the suboesophageal ganglion.

ii) Nerve fibers originating in the suboesophageal ganglion and ending in the cerebral ganglion.

iii) Nerve fibers beginning from the suboesophageal ganglion or from more caudal ganglia and passing through the cerebral ganglion.

Some of them go to the periphery by way of cerebral nerve trunks (Fig. 23, B), while others enter the enteric nerves and end in the thickening of the latter (Fig. 24, B).

B)

i) Sensory fibers coming from the epidermis by way of cerebral nerve trunks and ending in the suboesophageal or more caudal ganglia.

ii) Sensory fibers entering the suboesophageal or more caudal ganglia through the segmental nerve trunks and ending in the neuropile of the cerebral ganglion.

Besides, the circumoesophageal connective contains nerve cells, which

are bipolar or tripolar and resemble the intermuscular nerve cells in respect to the form, the stainability, and the course of axon. They seem to be identical with the cells described by KRAWANY in his Tafel V, Figs. 5 and 6. One of them tripolar in shape is shown in my Fig. 25, A; one axon of it ends in the cerebral ganglion, another in the suboesophageal ganglion, while the third axon enters the cerebral nerve trunk.

4) CEREBRAL GANGLION

The cerebral ganglion, located in the IIIrd segment, consists of two lobes, which are united by circumoesophageal connectives with the suboesophageal ganglion at their ends, and emits 10–11 pairs of cerebral nerve trunks from the frontal margin (Fig. 21), connecting with the enteric nerve thickening at its ventral side. So far as I am aware, the histology of this ganglion has never been reported on except by KRAWANY, who observed it in *Eisenia foetida*. In the present chapter I wish to compare the description of this author with my observation in *Pheretima communissima*.
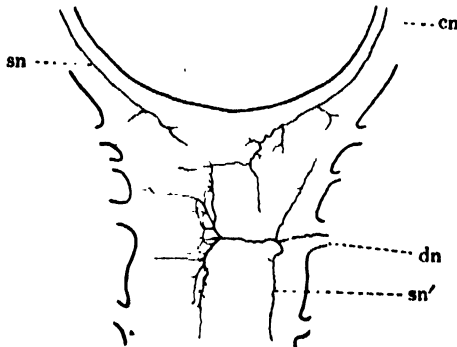

Fig. 20. The sensory nerve fibers in the suboesophageal ganglion coming through the circumoesophageal connectives from the cerebral ganglion in the 15 mm long embryo; ×90. cn=circumoesophageal connectives, dn= double nerve trunks, sn=sensory fibers coming through the circumoesophageal connectives, sn′=sensory fibers coming through the double nerve.

We find two parts in the cerebral ganglion, one of which is the cortical layer, where many nerve cells are arranged compactly, and the other represents the neuropile, situated in the interior and made by many intermoven nerve fibers. The neuropile is continuous with the corresponding part of the circumoesophageal connective and contains nerve fibers, which coming from various parts either pass or end there. On the other hand, in the cortical region we see, in addition to small nerve cells which are characteristic of this ganglion, greater ones such as those seen also in the ganglia of the ventral cord. Moreover, near the places, where the cerebral nerve trunks leave the ganglion, we find a great many small nerve cells, whose processes go into cerebral nerves. Below these various elements

will be described in separate paragraphs.

i) Characteristic nerve cells in the cerebral ganglion.

In the antero-dorsal part of the brain, a characteristic sort of small nerve cells are arranged in layers; KRAWANY calls them "Binnenzellen". These cells are easily stained vitally with methylene blue, probably owing to their position near the surface of the ganglion. Fig. 22 and Plate XVII show the characteristic nerve cells by surface views and cross sections of the cerebral ganglion. The cells in question seem to be identical with the cells drawn by KRAWANY in his Tafel IV, Figs. 5-8. As Fig. 22 indicates, most of them are multipolar, but the others are bipolar or tripolar, and the number of them in the newly born worm is
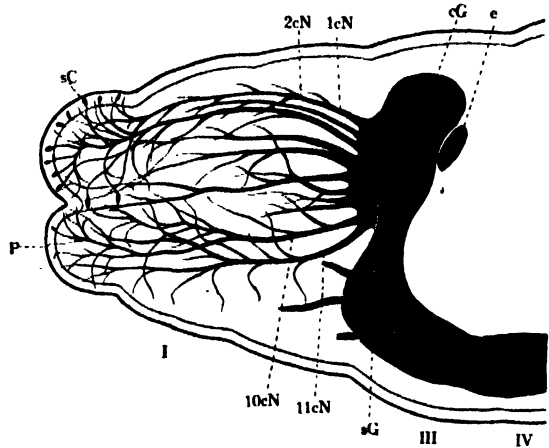

Fig. 21. The distribution of the cerebral nerve trunks in the left half of the anterior segments of the 20 mm long embryo; ×40. 1cN—11cN=from the 1st cerebral nerve trunk to 11th cerebral nerve trunk, cG=cerebral ganglion, e=enteric nerve, sC=sensory cells, sG=suboesophageal ganglion, P=prostomium, I-IV=No. of the segment.

about 100, making 1/10 of the total number of ganglion cells contained in the cerebral ganglion. They are very much increased in the adult stage, amounting to more than half of all the nerve cells possessed by this ganglion. According to KRAWANY's estimation, about 500 cells of this type were found in *Eisenia foetida*, but, to my regret, he did not report the total number of nerve cells contained in the cerebral ganglion. When treated with DELAFIELD's haematoxylin and eosin or HEIDENHAIN's haematoxylin, they differ also in stainability from other nerve cells. In the former method they show special affinity to eosin, and they are stained more deeply than other ganglion cells by DELAFIELD's haematoxylin and eosin (Fig. 23, C). Besides, by MALLORY's method they take a more reddish purple coloration than the others. Their form is also somewhat different and the nucleus is relatively small in comparison to the cell body. As seen in Fig. 22 and Plate XVII, their form is very special, having

short processes which appear as fine varicose fibers. While examining these nerve cells, I saw one region which is characterized by the presence
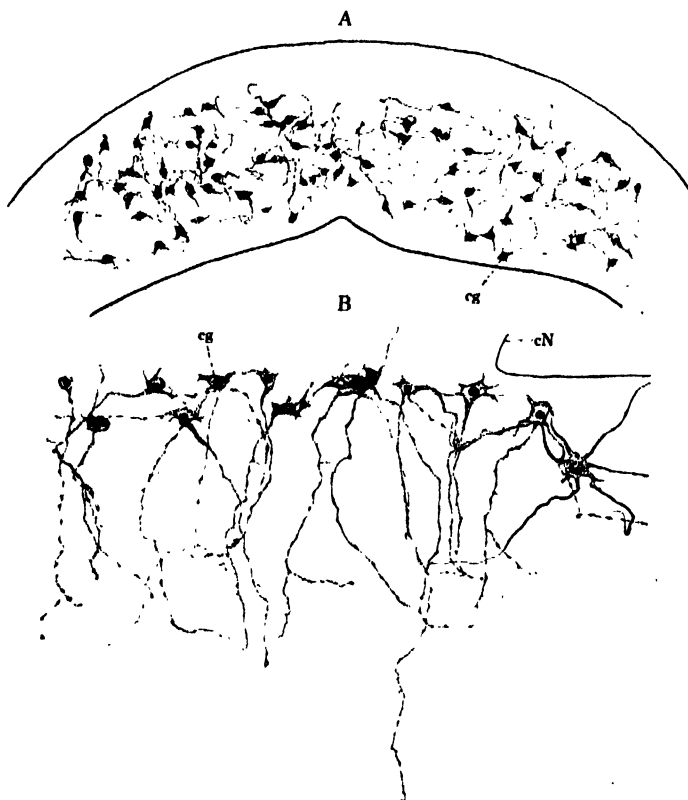

Fig. 22. The characteristic nerve cells in the dorsal layer of the cerebral ganglion. A) The surface view of the cerebral ganglion in the newly born worm; ×100. B) The cross section view of the cerebral ganglion in the 10 weeks old worm; ×350. cg=characteristic ganglion cells, cN=cerebral nerve trunks.

of a network covering the dorsal border of the neuropile in the cerebral ganglion (Fig. 23, C and Plate XVII). This network is made of the above mentioned varicose fibers, as KRAWANY ('05) also emphasized this feature. I did not, however, observe the same relation as indicated in his Tafel IV, Fig. 12, but I found only the cells such as shown in Tafel IV, Fig. 8 of his work. There is a tendency for the processes of these cells to terminate only at the neuropile, without extending to the far region as the processes of other gross ganglion cells do. The formation of this

network is perhaps the unique nature of the nerve cells in question; in other words, it must be looked upon as one of the chatacteristica of the
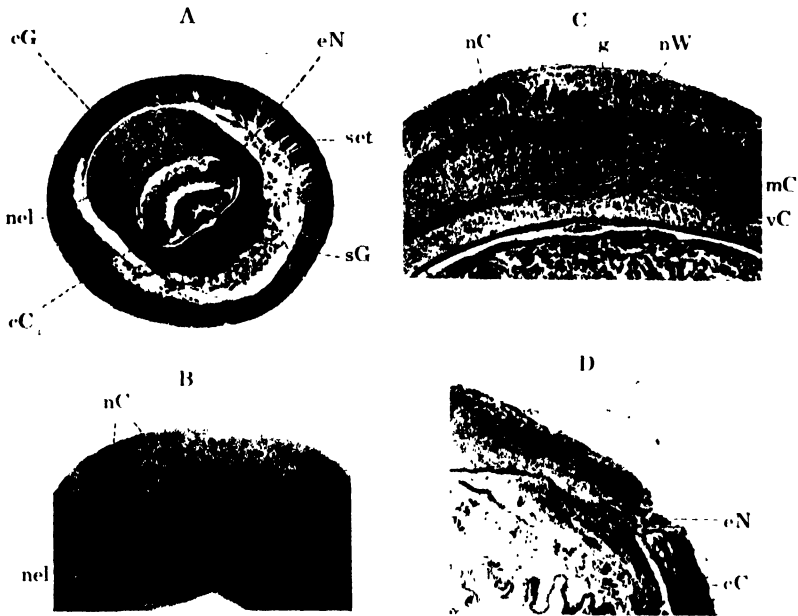

Fig. 23. The photographs of the cerebral ganglion. A) Cerebral ganglion in the cross section of the newly born worm by haematoxylin staining; ×30. B) The characteristic small nerve cells in the dorsal cell layer in the cerebral ganglion of the newly born worm by methylene blue staining; ×75. C) The characteristic small ganglion cells in the cerebral ganglion of the 5 weeks old worm by haematoxylin staining; ×70. D) The enteric nerve thickening in the full-grown worm; ×35. nC = characteristic small ganglion cells, eN = enteric nerve thickening, nel = neuropile in the cerebral ganglion. cC = circumoesophageal connectives, g = larger ganglion cells, cG = cerebral ganglion, sG = suboesophageal ganglion, nW = network covering the neuropile, mC = "mittlere Querkommissur", vC = "hintere Querkommissur", set = setae.

cerebral ganglion. But the function of these cells could not be determined, as special physiological experiments were not undertaken in this work. In the future I hope to discover, whether the cells of this type are present also in other Annelida besides the earthworm.

ii) *Larger ganglion cells.*

In addition to the characteristic nerve cells just mentioned above, the cerebral ganglion contains also many larger ganglion cells, which resemble the cells in the suboesophageal ganglion or in the ganglia of the ventral cord. Two types may be distinguished the so-called " Schaltzellen " and

the motor cells, which is also the case in the other ganglia. The former
are located in the ventral portion of the ganglion and their axons terminate
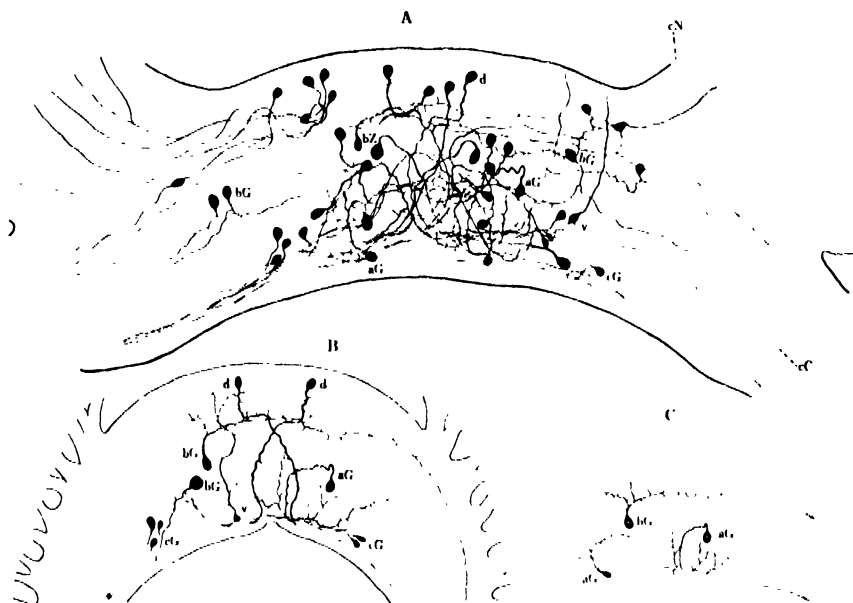in the neuropile of the ganglion, while the latter mostly lie in the dorsal

Fig. 24. The neurons in the cerebral ganglion; × 80. A) Various kinds of
neurons in the 8 weeks old worm. B) and C) The courses of some neurons in
the newly born worm. aG = associative ganglion cells, bG the neurons sending
their axons into the circumoesophageal connectives in the dorsal cell-layer, cC —
circumoesophageal connectives, cG = small ganglion cells sending their axons into
the circumoesophageal connectives in the ventral side, cN = cerebral nerve trunks,
d - neurons in the dorsal region sending their axons to the ventral portion, v =
neurons in the ventral part sending their axons to the dorsal portion.

layer and their axons go out to the head region through the cerebral
nerve trunks. The so-called "Schaltzellen" were described by KRAWANY
as "Binnenzellen" in the cerebral ganglion (Tafel IV, Bz₁ and Bz₂). Some
of these nerve cells seem to be pseudo-unipolar, their processes dividing
into two branches immediately after leaving the cell-body, and one of them
extends to the periphery by way of the nerve trunks or the circumoe-
sophageal connective, while the other ends with small terminal branches in
the neuropile of the cerebral ganglion, as seen in Fig. 24 (bZ). According
to the monograph by HANSTRÖM ('28) a similar type of nerve cells exists
also in some other worms, for instance, in *Nereis* (HOLMGREN, '16), and

in *Glycer* (HANSTRÖM, '28). Therefore, these cells may be thought of as characteristic of the cerebral ganglion of Annelida.

In the ventral part of the cerebral ganglion we see also the unipolar cells similar to those which were designated by KRAWANY as "Us" and "Ns" in his Tafel IV, Fig. 9. The former "Us" send their processes through the midline along the circumoesophageal connective of the opposite side (Fig. 24, B, bG), while the axons of the latter without crossing go into the connective of the same side (Fig. 24, B, cG). Moreover, these ganglion cells may be classified into two types, one of which sends its process in the neuropile of the cerebral ganglion, terminating there after being divided into many collaterals, while the other gives off the process to the suboesophageal or the neighbouring ganglia by way of the circum-oesophageal connective. On the other hand, in the dorsal layer of the ganglion concerned, another kind of cells is found, whose axons go over to the ventral side (Fig. 24, B d), while cells just the reverse of these are also seen, namely those, which lie in the ventral part and send their axons to the dorsal portion of the cerebral ganglion (Fig. 24, B v).

iii) Small nerve cells in the cerebral ganglion.

In Fig. 25, is shown a group of small ganglion cells which are localiz-ed near the anterior border of the cerebral ganglion, and give off their axons into the cerebral nerve trunks. They seem to be of a type similar to the cells demonstrated by KRAWANY in his Tafel V, Fig. 1. He stated that these cells appear to send their processes mainly in the cerebral nerve trunks, but he did not succeed in tracing their courses thus far owing to the failure of the staining. As seen in Fig. 25, they are smaller and unipolar, having a long axon, which is devoid of small collaterals. Upon examining many preparations, I could further determine three different courses of the processes, as follows : — In one of them the axon is directed toward the neuropile of the cerebral ganglion ; the second sort óf cells send their processes to the suboesophageal ganglion through the circumoesophageal connective ; and the third kind gives off their axons to the enteric thickening. In short, also these cells may be characteristic of the cerebral ganglion, just as the two kinds of cells mentioned in the foregoing paragraphs, but they differ remarkably from the so-called characteristic ganglion cells in their axons, the latter having fine varicose processes.

iv) Neuropile in the cerebral ganglion.

In the cerebral ganglion, three different parts are discernable in the neuropile unlike those of the ventral cord. According to KRAWANY,

these parts are named as " vordere, mittlere, und hintere Querkommissur ".
I should like, however, to designate them as " anterior network " instead
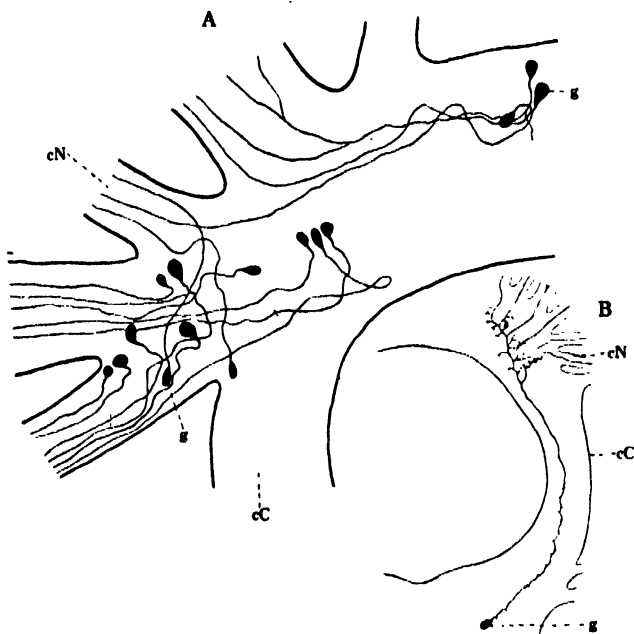
Fig. 25. The neurons sending their axons into the cerebral
nerve truuks in the 10 weeks old worms; ×100. A) The neurons
in the cerebral ganglion. B) The neurons in the suboesophageal
ganglion, cC = circumoesophageal connective, cN = cerebral nerve
trunks, g = small nerve cells.

of " vordere Querkommissur " owing to the actual appearance of the
regions.

The first of these, namely, anterior network, which covers the dorsal
surface of the whole neuropile, shows the character, that it consists of
fine varicose nerve fibers of the so-called characteristic ganglion cells
(KRAWANY's " kleine Zellen ").

The second part, " mittlere Querkommissur ", which is localized in the
middle portion of this neuropile, looks somewhat similar to the neuropile
of the ventral cord containing the terminal ends of various nerve fibers;
some of the latter are afferent nerve fibers entering through the cerebral
nerve trunks, and through the segmental nerves of the ventral cord.

The third portion " hintere Querkommissur ", which exists in the
ventral side of this neuropile, indicates a strand of coarse nerve fibers;

masses of the fibers show an elevation dorsally in the middle portion of the strand; this relation is seen in Fig. 26, A. A similar condition was demonstrated already by KRAWANY in Tafel IV, Fig. 9.

The third portion is built of the processes of the "Binnenzellen" or "Schaltzellen", which are related to the motor nerve cells (Fig. 23, B and 26 A). According to my observation most of the nerve fibers which
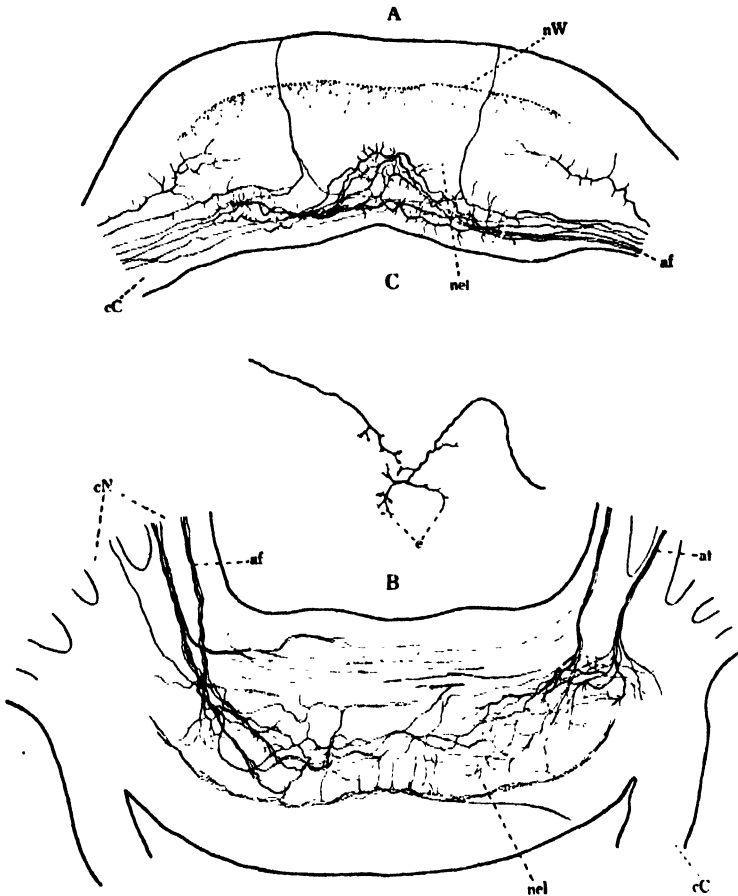
Fig. 26. The afferent nerve fibers in the cerebral ganglion. A) The afferent nerve fibers coming through the circumoesophageal connective in the 15 mm long embryo; ×100. B) The afferent nerve fibers coming from the cerebral nerve trunks in the newly born worm; ×100. C) The nerve ending in the cerebral ganglion in the newly born worm; ×350. af=afferent nerve fibers, cC=circumoesophageal connectives, cN=cerebral nerve trunks, e=nerve ending, nel=neuropile, nW=anterior network covering the dorsal surface of the neuropile in the cerebral ganglion.

constitute these bundles have entered from the circumoesophageal connectives, and they give off small collaterals in their courses. As shown in Fig. 26, C, we see in this neuropile the similar manner of endings of the nerve fibers as in other parts of the nervous system.

v) Cerebral nerve trunks.

In *Pheretima communissima*, about ten cerebral nerve trunks arise from the antero-lateral border of the cerebral ganglion on each side bilateral symmetrically. They are grouped usually in five (OGAWA, '34, Fig. 2). As Fig. 21 shows, most of the cerebral nerve trunks are supplied to the prostomium, the wall of the buccal cavity, and the other head regions. The details of these innervations was studied by IMAI ('28) in *Ph. megascolidioides*, but in his article the nature of the nerve fibers passing through the cerebral nerve trunks was not especially reported. My previous observation in the cross sections of the material stained with haematoxylin shows that there are two kinds of nerve fibers in these nerve trunks; there the coarse nerve fibers such as usually to be found in all segmental nerve trunks are absent, and only ordinary sensory nerve fibers and small efferent ones are found.

From the foregoing remarks I wish to summarize here the character of the nerve fibers passing through the cerebral nerve trunks as follows: Concerning the efferent fibers, the first group arises in the suboesophageal ganglion, passes through the circumoesophageal connective, and is supplied to the body wall of the head region, giving off many branches at the border of the cerebral ganglion (Fig. 25, B). The second group originates from numerous small nerve cells localized in the lateral border of the cerebral ganglion, and go into the cerebral nerve trunks without branching (Fig. 25, A). The third group starts from the dorsal layer of the cerebral ganglion, and leaves this ganglion by way of the cerebral nerve trunks, issuing sometimes many branches. On the other hand, as to the afferent nerve fibers, some of them seem to arise from the epidermis of the head region, and terminate in the neuropile of the cerebral or suboesophageal ganglion after they have passed through the cerebral nerve trunks, while the others are given off from the sensory cells in the wall of the buccal cavity. However, I could recognize with certainty only the sensory nerve fibers, which terminate in the neuropile of the cerebral ganglion, as shown in Fig. 26, B.

vi) Enteric nervous system.

A pair of ganglionic thickenings are found along the ventro-lateral border of the cerebral ganglion. They innervate the walls of the diges-

tive organs with about six nerve trunks, as already described by HESS ('24) in *Lumbricus*. According to my calculation of cells, the enteric ganglionic thickening of the newly born worm is relatively larger than that of the adult (Fig. 23, A and D), and the number of ganglion cells contained in this thickening amounts already in the newly born worm to the full number of these in the adult. So far as I am aware, no detailed studies have ever been made on the histological structure of the enteric nervous system; especially the relation between these nerves and the ganglion cells in the cerebral or suboesophageal ganglion lies for the most part unsettled. So I tried in this work to solve the above mentioned problems by observing the newly born worms and the old embryos stained vitally with methylene blue.

As shown in Fig. 23, A and D, there are found two structurally different parts in each thickening; one part is the outer layer containing many ganglion cells, while the other is the neuropile-like middle region, which appears as a network of fine interlacing nerve fibers.

In addition, concerning the course of nerve fibers in this region, the following facts have been ascertained; the first type of the fibers arises from the ganglion cells in the suboesophageal ganglion, and terminates in this thickening after passing through the circumoesophageal connective, as this relation is shown in Fig. 27, B; the second sort of fibers originates in the cerebral ganglion, and terminates in this thickening (Fig. 27, A); the third type originates in the ganglion cells situated in the cerebral ganglion and reaches to the wall of the alimentary organs (Fig. 27, A). Moreover, we found in the enteric nerve trunks some afferent fibers entering there from the wall of the alimentary tract, and terminating in this thickening or in the cerebral ganglion (Fig. 27, C).

5) INTERMUSCULAR NERVE CELLS

Among the earlier scientists, DAWSON ('21) studied concerning the size, form and distribution of the intermuscular nerve cells in the earthworm and noted their relation to the peripheral nervous system. In general they are considered as very important from the view-point of phylogeny, inasmuch as they indicate the transformation of the nerve net of the lower invertebrates (*Coelenterata* etc.) into the centralized deeply-ing system of the higher invertebrata (for instance, *Annelida*). It seems equally as logical to look upon them as scattered ganglion cells or motor cells of the primitive nerve net which have not yet been in-

corporated in the ventral nerve cord. The distribution and arrangement of these cells may give us some hints on the functional explanation of
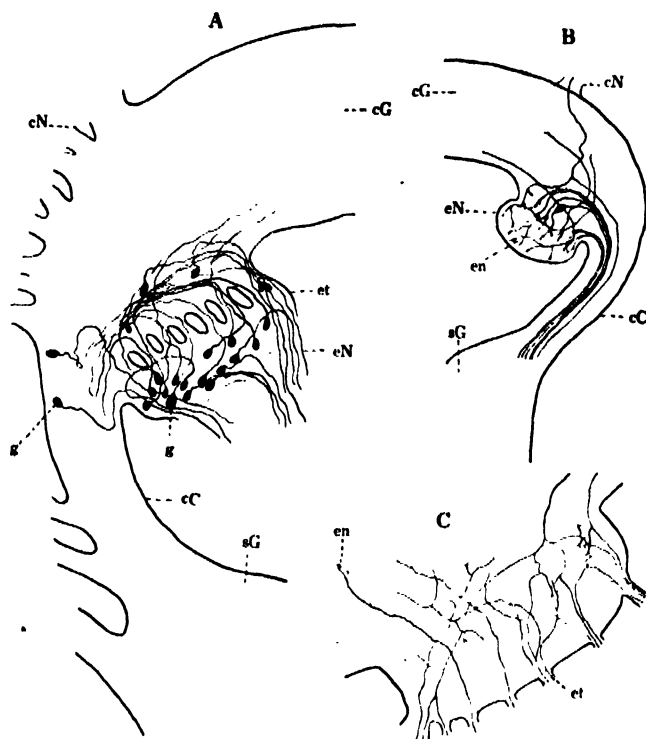
Fig. 27. The neurons and nerve fibers in the enteric nerve thickening. A) The neurons sending out their axons into the cerebral ganglion or enteric nerve trunks in the newly born worm; ×100. B) The nerve fibers coming from the suboesophageal ganglion, and terminating in the enteric nerve thickening in the 10 mm long embryo; ×100. C) The afferent fibers coming through the enteric nerve trunks from alimentary organs in the newly born worm; ×200. cC=circumoesophageal connectives, cG=cerebral ganglion, cN=cerebral nerve trunks, eN=enteric nerve thickening, en=nerve ending, et=enteric nerve trunks, g=ganglion cells in the enteric nerve thickening, g′=ganglion cells in the cerebral ganglion sending their axons into the enteric nerve, sG=suboesophageal ganglion.

these cells. Since, however, there have been no records relating to these cells in embryos or young worms, I intend in the present research to determine their distribution, arrangement and relation to the peripheral nervous system in the younger stages of the worm. In practice, I have observed them by surface and cross sectioned views in the preparations

stained intravitally with methylene blue. In the embryo or young worms (1 or 2 weeks after birth) these cells appear clear and conspicuous, being
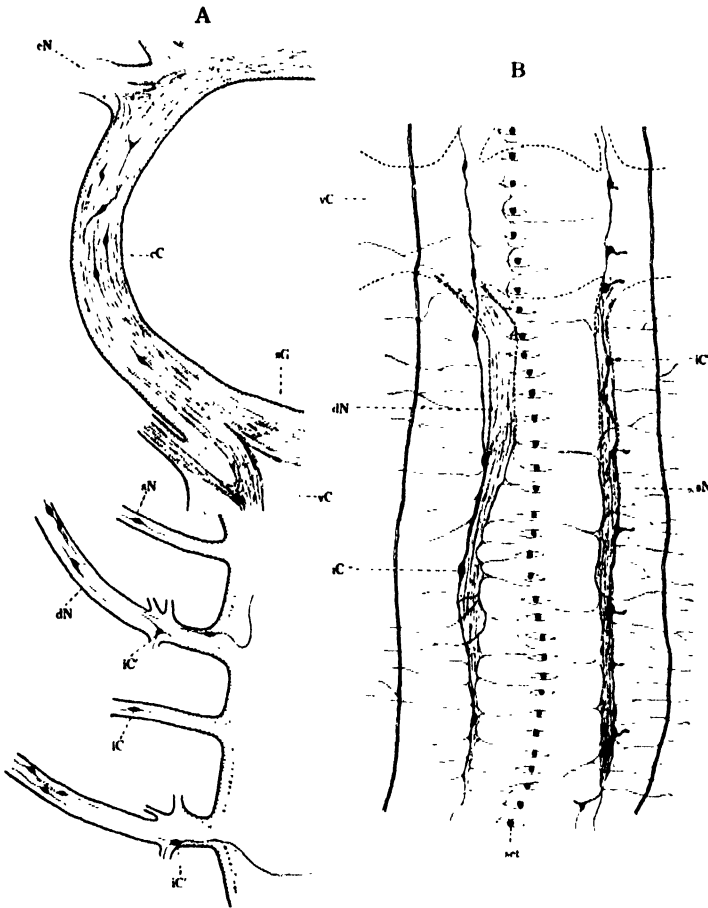
Fig. 28. The distribution of the intermuscular nerve cells. A) The intermuscular nerve cells in the circumoesophageal connectives and the segmental nerve trunks of the 8 weeks old worm; ×32. B) The intermuscular nerve cells along the segmental nerve trunks, by the surface view, in the 15 mm long embryo; ×80. cC=circumoesophageal connective, cN=cerebral nerve trunks, dN=double nerve trunks, iC=bipolar intermuscular nerve cells, iC'=tripolar or multipolar intermuscular nerve cells, sG=suboesophageal ganglion, set=setae, sN=single nerve trunk, vC=ventral cord.

recognized easily by the surface view of the worm-body, while in the adult, viz. larger worms (10 weeks or more after hatching) the surface view does not give the clear aspect of them and their existence can be

ascertained only in the cross sections.

I wish here to classify the intermuscular nerve cells by their morphological character and localization into three kinds: (i) cells in the interspace between circular and longitudinal muscle layers, (ii) cells in the segmental nerve trunks and circumpharyngeal connective, (iii) cells in the circular muscle layer beneath the epidermis.

   i) *Intermuscular nerve cells lying in the interspace between the circular and longitudinal muscle layers.*

In the embryo, these cells arrange themselves regularly in two rows along the two segmental nerve trunks (double and single nerves) in each segment. One row belongs to the single nerve, while the other is connected with either anterior or posterior trunk of the double nerve (Fig. 28, B and Fig. 30, B). Also the number of these cells seems to be definite, in the former (double nerve) 18, in the latter (single nerve) 20. In the cells belonging to the single nerve, the shape and the relation of the epidermis to them are interesting, as seen in the cross section view (Fig. 29); they are tripolar (Fig. 29, B) or quadripolar (Fig. 29, C), and are uniformly found at the bifurcation of the single nerve trunk, where the dorsal and ventral ramification of the nerve rings branch off.

The cells with four processes (Fig. 29, C) are situated in the ventral side where the single nerve ring (Plate XVI, A) does not yet enter the interspace between circular and longitudinal muscle layers, making bud-like termination, just as the epidermal sensory cells (Fig. 29, C, en), while the main axon passes distally through the longitudinal muscle layer, and goes proximally towards the ventral cord, combining itself with the single nerve (Plate XVI, A); on the other hand, two other processes, passing dorsally or ventrally along the space between the two muscle layers mentioned above, terminate at last in the longitudinal muscles. Furthermore, the tripolar cells lie in the space between two muscle layers where the single nerve passes through dorsally. Of the three processes (Fig. 29, B), the first one terminates in the epidermis, like that of the quadripolar cells, the second one passes proximally towards the ventral cord by way of the single nerve, and the third one goes distally in the mid-dorsal direction, and finally terminates in the longitudinal muscle. As seen in Plate XVI, A and B, in all cases the lateral processes of these cells were observed running parallel with the nerve ring, sometimes being connected to each other. In the young worm (10 weeks after hatching), these cells are not so clearly seen owing to the growth of other tissues, although they exist in the same region as in the embryos. The development

of the termination in the epidermal layer varies from the bud-like to the stick-like shape (Fig. 29, B).
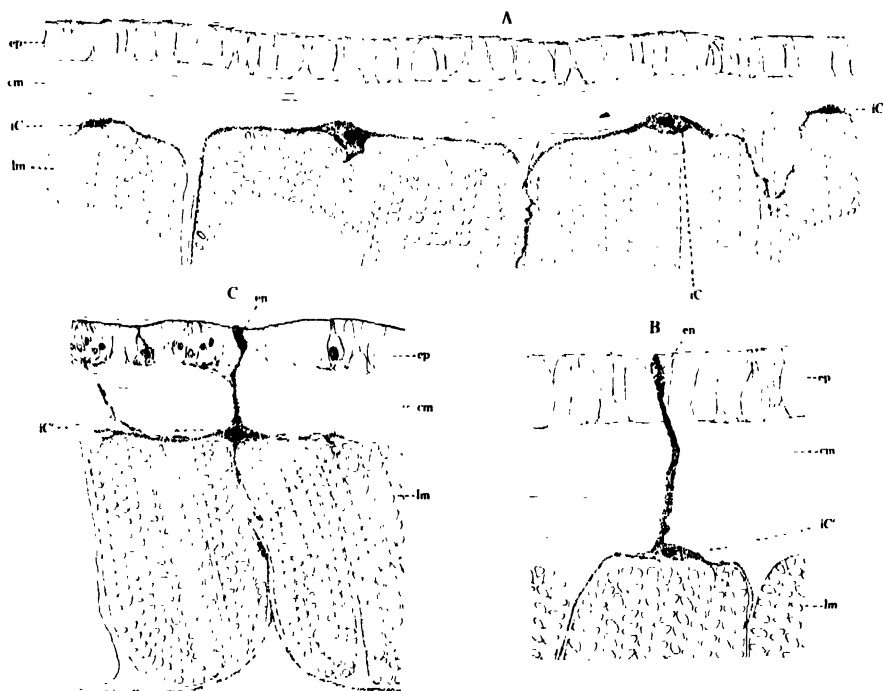
Ffg. 29. The intermuscular nerve cells in the interspace between the circular and longitudinal muscle layers, by the cross section through the segmental nerve trunks; ×320. A) The intermuscular nerve cells belonging to the double nerve trunk in the 10 weeks old worm. B) and C) The intermuscular nerve cells belonging to the single nerve trunk in the 10 weeks old worm (B), and the newly born worm (C). cm = circular muscle layer, en - ending in the epidermis, ep—epidermis, iC - bipolar intermuscular nerve cells, iC′—multipolar intermuscular nerve cells, lm -longitudinal muscle layer.

The other row (Plate XVI, B) of the intermuscular nerve cells makes up a ring along the course of the double nerve, consisting of spindle-shaped, bipolar or tripolar cells. These cells, unlike the cells belonging to the single nerve, are not so regular in the distribution of their processes. One of their two or three processes extends proximally towards the ventral cord by the single nerve, while the others pass distally towards either the middorsol or midventral line, and lastly terminate in longitudinal muscles. The spindle-shaped bipolar cells exhibit some striking variations in size (Fig. 29, A), as already mentioned by DAWSON

(Figs. 3 and 4). According to von Szüts, smaller bipolar cells, which were called by him merely as " die Nervenzellen ", contain neurofibrils which, although often highly branched, transverse the cell-bodies without anastomosing, while larger bipolar cells show the intracellular fibrils anastomosing to form complex networks. These latter were given the name of " die intermuskulären sensorischen Ganglienzellen ".

From my preparation, I could not definitely ascertain the character of these cells, but some differences were found as to the morphological character or the course of their processes. According to my opinion, the small cells are more abundant in the ventral side than in the other parts. But it is difficult to decide whether their processes enter the anterior trunk of the double nerve or the posterior trunk. In general, as shown in Fig. 30 and Plate XVl, A, 1 found that the intermuscular nerve cells which project their receptor into the epidermis belong to the single nerve, which has few sensory nerve fibers, while the cells which lack such receptors belong to the double nerve, especially to its anterior trunk, which is occupied by numerous sensory nerve fibers.

    ii) Intermuscular nerve cells in the nerve rings and circumpharyngeal connective.

Langdon ('05) first described the presence of cells in the three nerve rings in several segments and determined further the number of them in

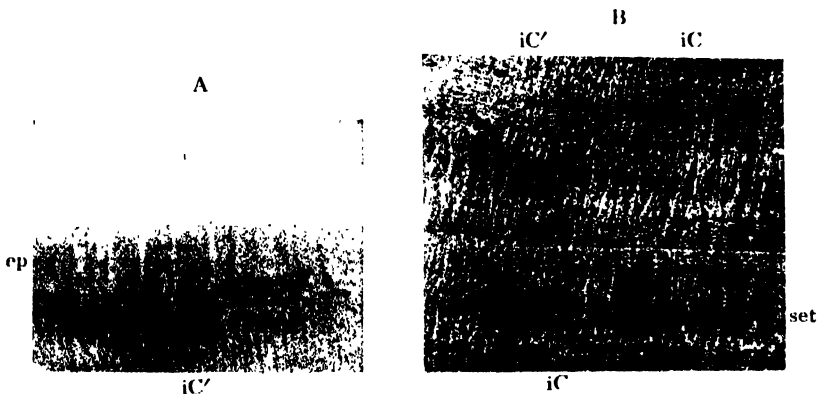Fig. 30. The intermuscular nerve cells belonging to the segmental nerve trunks by photographs. A) The tripolar intermuscular nerve cells belonging to the single nerve trunks, (newly born worm); ×120. B) The distribution of the intermuscular nerve cells along the segmental nerves in the newly born worm; ×90. ep—epidermis, iC – bipolar intermuscular nerve cells, iC′ tripolar intermuscular nerve cells, set setae.

every nerve ring. All these cells were said to be of the bipolar and tripolar type. DECHANT ('06) succeeded by the intravitam methylene blue method in demonstrating these ganglion cells in the nerve ring. In the anterior and posterior nerve trunks of the double nerve, he found the ganglion cells regularly arranged, usually in groups of three, in the region of the dorsal pair of setae. And he recognized in them two types of cells, one of which is spindle-shaped or bipolar, while the other is tripolar or triangular. In 1909, KOWALSKI described tripolar cells by the neurofibrils method of RAMON Y CAJAL. In my preparation (worms 8 weeks after hatching), treated by the intravitam methylene blue method, regularly arranged ganglion cells were found, as shown in Fig. 28, A, in the single and double nerves. In the double nerve ring, tripolar cells are found at the bifurcation nearest to the ventral cord. One of their processes extends proximally in the double nerve trunk, while two others pass distally, one in the dorsal, and the other in the ventral side of the body issuing short branches towards the epidermis. These cells are also represented in sections by the haematoxylin method in my previous figure (OGAWA, '30). More distally the intermuscular cells are arranged in three groups, one of which has tripolar cells, and one of the two others has small spindle-shaped cells, while the remaining one has large cells of the same shape. One process of these cells passes proximally towards the ventral cord, while the other goes distally towards the middorsal line. The peripheral course of the processes could not be further determined. In the single nerve these cells are very few; only one bipolar cell was observed at the bifurcation of the lateral branch, the same as was noted in DAWSON's work. The cells in question are more abundant in the anterior portion of the worm body than in the typical segments. Fig. 28, A indicates the cells in the circumpharyngeal connective, in which several cells of spindle-shaped or tripolar types are present. One of the processes of the tripolar type extends towards the cerebral ganglion, one passes to the suboesophageal ganglion, and the third process proceeds distally in the cerebral nerve trunk. So far as I am aware, no authors have yet described the above mentioned direct relation between the intermuscular nerve cells and the cerebral ganglion.

iii) *Intermuscular nerve cells in the baseepiment.*

Crescent-shaped bipolar cells were observed in large numbers in the baseepiment which is just under the epidermis. These cells are quite different from any other intermuscular cells already described. They could be clearly seen, when we employed, at first, the intravitam and,

afterwards, the submersion method with methylene blue. Moreover, they appear only in preparations in which the sense organs of the epidermis were selectively stained just as DAWSON said. The one process of them is directed outward, while the other is lost eventually in the baseepimental
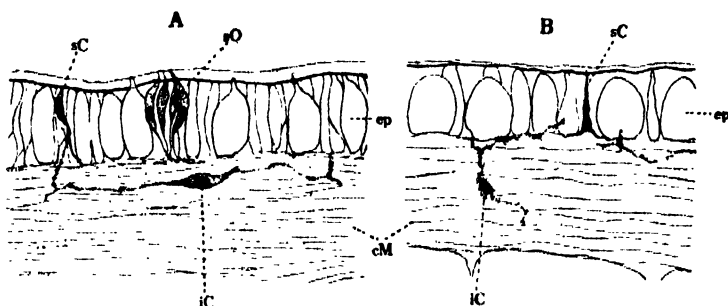
Fig. 31. The intermuscular nerve cells in the baseepiment by the cross section view. A) The intermuscular nerve cells giving off their processes into the epidermis in the 8 weeks old worm; ×400. B) The intermuscular narve cells giving off their axons into the epidermis and muscle fibers, in the 5 weeks old worm; ×350. ep= epidermis, iC=intermuscular nerve cells, cM=circular muscle layer, sC=sensory cells, sO=sense organs.

network (Fig. 31). They resemble in some extent the crescent-shaped tripolar cells described by DAWSON, though there are some differences in details of the structure from those described by this author. In DECHANT's figure ('06) many bipolar nerve cells are seen in the baseepimental network; some of them are connected with each other by means of their axons. From my observation the processes of the sensory cells in the epidermis and of the baseepiment seem to form the subepidermal network. I have observed almost all of the features reported by DAWSON and DECHANT, except that I could not find that their axons are ever connected. Moreover, I could not find long axons, but only two short axons issuing from them. By using the methylene blue method it is generally found that if the motor cells of the ventral cord and the intermuscular nerve cells are brightly stained, the epidermal sensory cells take the dye very faintly. This intermuscular nerve cell is very small in size and in this respect resemble the sensory cells in the epidermis. They are to some extent similar in size and form; also they are distributed over the whole surface of the body. From these circumstances we are led to assume that the intermuscular cells in the baseepiment may serve for the conduction of the sensory impulse from the epidermis. In addition I have directed my attention to the problem concerning the relation of these cells to other

elements; for instance, whether they are connected with the receptor or with the effector portion of the nervous system. We see above all the intimate relation between them and the setae or gland cells in the epidermis; the setae are surrounded by them or connected with them. At the same time the others of their axons are found to terminate in the network.

iv) The intermuscular nerve cells in the early embryonal stage.

The intermuscular nerve cells seem to play rather an important part in the early stage of the embryo. As seen in Plate XVI, C and D, they already appear in the very small embryo which has only about ten segments and is 0.5 mm in length. At this period of development the intermuscular nerve cells are arranged segmentally in the ventral side of the worm body, as if they represented the segmental nerve trunks, but in my opinion, the latter have not developed at all in this stage; the ventral nerve cord has only begun to appear in the head and middle portions. To my regret, I can not ascertain the relation between these intermuscular nerve cells and the central nervous system, which has just
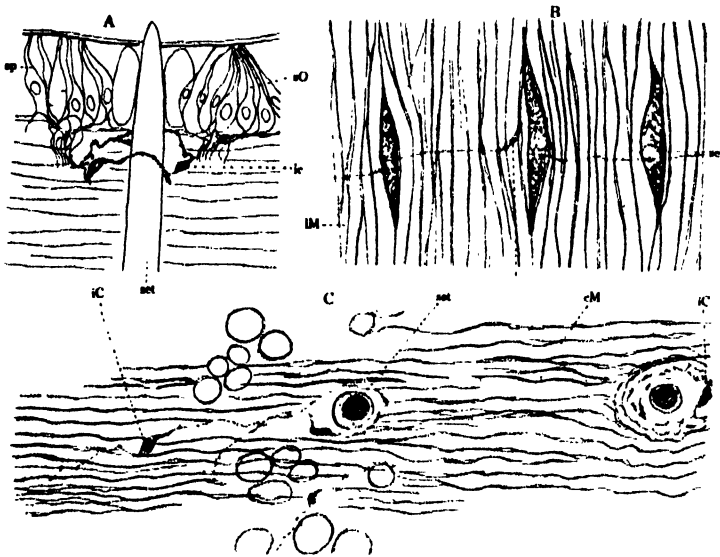
Fig. 32. The correlation of the intermuscular nerve cells and the setae. A) The intermuscular nerve cells in the cross section of the 8 weeks old worm; ×320. B) The intermuscular nerve cells in the surface view through the longitudinal muscle of the newly born worm; ×280. C) The intermuscular nerve cells in the surface view through the circular muscle layer of the 4 weeks old worm; ×320. cM=circular muscle layer, iC=intermuscular nerve cells, ep=epidermis, lM=longitudinal muscle layer, set=setae, sO=sense organs.

appeared. At any rate, the intermuscular nerve cells, which are situated along the segmental nerve trunks in the adult worm, seem to develop earlier than any other part of the nervous system, suggesting that they enter to function before the central nervous system begins to work. Moreover, the other sort of intermuscular nerve cells, i. e. those existing .in the baseepiment also already appear in the early embryonal stage in a diffused condition. From these observations it may be assumed that the intermuscular nerve cells indicate the state of transition from the diffused nervous system of Coelenterata to the more centralized system of Annelida. The earliest development, or the origin of the intermuscular nerve cells could not be determined in the present research.

6) THE SENSORY NERVE CELLS IN THE EPIDERMIS.

i) *Sense organs and sensory cells.*

To compare them with the motor cells, sensory cells in the epidermis were also observed by the haematoxylin and methylene blue methods. In my observations, worms of various periods were used as the materials, and the observation was for the most part carried on by the surface view after submersion of the materials in methylene blue solution, but sometimes, cross sections were also observed after treatment with both above mentioned dyes. Many authors, for example, LEYDIG ('65), UDE ('86), CERFONTAINE ('90), LENHOSSÉK ('92) have described the epidermal sensory cells, which bear hairs at their peripheral ends and are frequently found in small groups surrounding a pore canal. Especially, R. HESSE ('94) on *Lumbricus herculus* and *Allolobophora chloratica*, and LANGDON ('95) on *L. agricola*, have reported in detail upon sense organs in the epidermis. In my preparation, there are found two kinds of sensory nerve cells; one of them represents solitary sensory cells, which are scattered all over the epidermis, while the other is the cells grouped into the special sense organs.

In regard to the former, there are some differences of opinion among the authors; KULAGIN ('88) and LENHOSSÉK ('92) observed, that each of the solitary sensory cells has a hair projecting through the cuticule and the base connected with a nerve fiber; also RETZIUS ('92) figured and described the cells almost in the same way as LENHOSSÉK, while recently SMALLWOOD ('30) has given the description of a new type of isolated sensory nerve cells in the epidermis.

In my preparations, the isolated sensory nerve cells are abundantly

found, scattered between the sense organs, in all individuals examined. They can be distinguished from the cells of the sense organs by their distribution and form (Fig. 33), as they are arranged at right angles to the base membrane, their processes running for a short distance just beneath the base epiment, turning sometimes towards the segmental nerve ring through the layers of the muscles. In the surface view, they are seen in networks, as shown in Fig. 33, B. In the prostomium and in the first metamere, the sense organs are densely

Fig. 33. The solitary sensory cells; ×400. A) The sensory cells in the epidermis of the newly born worm by the cross section view. B) The distribution of the sensory cells in the epidermis of the 8 weeks old worm by the surface view. cM = circular muscle layer, ep = epidermis, sC = solitary sensory cells.

crowded on the whole epidermis, and in the anal metameres also they are more numerous than in the typical segments. In the haematoxylin preparation the form of each sense organ is in general ovoid, the smaller end of which projects into a raised spot in the cuticule covering above it, while the broader end is flattened and rests on the base membrane of the epidermis. The ovoid form may be broad or narrow, being usually more or less irregular.

On the other hand, in the typical segments they show the arrangement in three definite rows which are band-like and made up of numerous large sense organs; one row is in a line with the setae and may be called the middle zone, while two other rows are somewhat irregularly built up in the anterior and posterior regions of a segment. Moreover it may be worth while to note that in *Pheretima* the nephridial openings are arranged also in three rows where the sense organs are numerous.

Since the important organs such as, nephridium and setae, are located in the middle zone, it may be reasonable that sense organs are also accumulated in the same region. Miss LANGDON ('95) counted the number of sense organs in various metameres of *Lumbricus*. According to my observation in surface and cross sectioned preparations, the number of them are smaller than LANGDON's result, when compared in the corresponding segments.

The sensory cells occupy the greater part of a sense organ; 35–45 of them are counted in a large organ in the full grown worm, while there were only 18 in the smallest one. In the newly born worm, the largest organ has 10–18 cells, and the smallest one has about 7 cells.

If we observe this organ under the microscope, the sensory cells are found to send the main processes, which form a small bundle, through the circular muscular layer to the segmental nerve rings (Fig. 34). But
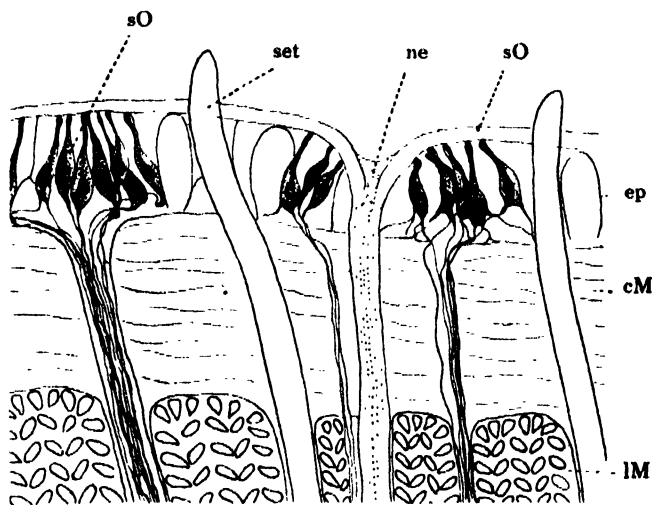
Fig. 34. The sensory organs in the epidermis of the 8 weeks old worm by the cross section through the setal line; ×400. cM=circular muscle layer, ep=epidermis, lM=longitudinal muscle layer, ne=nephridial canal, set=setae, sO=sense organ.

in the solitary sensory cells such formation of bundles was not seen. The sense organs increase in size and in number with the growth of the worm; in the typical segment the embryo has about 260 of the sense organs, while the worm 8 weeks after birth has about 302.

According to LANGDON ('95) the adult *Lumbricus* is said to have 700

sense organs.   To avoid repetition, a description of the number of nervous elements will be left until a later section.

ii) Distribution of sensory fibers in a ganglion.

The sensory fiber, upon entering a ganglion, usually takes the course similar to that described by earlier workers (LENHOSSÉK, RETZIUS); Fig. 12 shows this relation.   In the ganglion the main axon proceeds in two different directions, i. e. anterior and posterior, giving off numerous branches which bifurcate at their ends.   The most interesting feature of these branches lies perhaps in the fact that they topographically show a very intimate relation to the lateral giant fibers.   This fact suggests that some of the sensory fibers correlate closely with the giant fibers; this is already maintained by SMALLWOOD.   Some of them cross over the middle line and terminate in the neuropile of the opposite side.   Fig. 12 indicates the sensory fibers entering through the single and double nerve trunks in the typical segment.

iii) Photoreceptor nerve cells.

The so-called " Lichtzellen " or photoreceptor cells were also found by the two staining methods used in my study.   So far as the epidermis is concerned, the prostomium is most abundantly supplied with these cells, while the typical segments of the middle region of the body contain them in less number than in the segments described by HESS ('25).   In my preparations, they are generally not so numerous as one can expect from the results of HESS, who studied in details the structure, function, distribution, and number of these cells.   As to the structure and distribution I have found the relation almost identical with the observation of this author.   These cells are drawn in Fig. 35 by the cross

Fig. 35.   The photoreceptor cells in the prostomial epidermis of the 8 weeks old worm; ×400. ph=photoreceptor cell,   sO = sense organs.

section.   To my regret, I can not ascertain the connection between their processes and the other nervous elements.   Since the preparations were not very successfull in this observation, the exact number of photoreceptor nerve cells was not determined, but generally in embryos these cells are remarkably fewer in number than in larger worms.

V. THE NUMBER OF GANGLION CELLS, NERVE FIBERS AND OTHER CELLS CALCULATED DURING THE GROWTH FROM THE NEWLY BORN WORM TO THE ADULT

Some authors have hitherto spoken about the number of nerve cells, nerve fibers and other histological elements in the earthworm, as they were discussing about its nervous function. For example, the cells in the ganglia of the ventral cord in *Eisenia foetida* were enumerated by KRAWANY ('05), while the number of nerve fibers in the segmental nerve trunks in the same species was counted by SMALLWOOD ('30). I have also studied numerically the nerve cells and fibers in *Eisenia foetida*, but my results concerning the cells differ considerably from KRAWANY's data, although his method of calculation is not very clear, while the number of fibers estimated by me in segmental nerve trunks agrees quite well with SMALLWOOD's data (in my case 38–47, in his 38–52). Also in *Pheretima*, I have studied the number of nerve cells and fibers in the greater parts of the nervous system ('34). Furthermore, we must consider LENGDON's ('95) detailed studies on the sense organs and sensory nerve cells in *Lumbricus*, as this author at that time estimated the number of these organs and cells; HESS also calculated the photoreceptor cells in the whole body of *Lumbricus*. In 1926 SMALLWOOD published his study upon the number of muscle fibers in the circular and longitudinal muscle layers in connection with the problem of the innervation of peripheral nerves in *Eisenia foetida*.

Since I have previously found that the increase of nervous elements in the special segments, where sexual organs are present, is intimately correlated with the development of those organs, I wish in the present work to promote this problem further, calculating nerve cells and fibers in the principal parts of the nervous system at several periods during the growth. Moreover, in order to compare them, the other sorts of cells, muscle fibers, sensory cells in the epidermis, and other epidermal cells were also studied numerically.

At first, I classified the materials from the newly born worm to the adult in six stages as mentioned in the technique section.

The newly born worms exhibit almost the same organization as the adult ones except as to some genital organs. Since the structure of the worm body was already described in detail in the chapter of materials, I will consider in this chapter only the numerical change of cellular and fibrous elements during the growth. Tables II and III indicate the number of ganglion cells and nerve fibers in several parts

of the nervous system, while Charts II and III show the curves representing the increase of those elements. At the same time I took into consideration the change of the size of ganglion cells and nerve fibers at each stage of growth. The averages of the number are given with probable errors in each Table concerned.

1) Cerebral Ganglion and Cerebral Nerves

In the cerebral ganglion are gathered the greatest number of ganglion cells throughout the whole nervous system of the earthworm, and characteristic ganglion cells as mentioned already in the former chapter are found there abundantly. In the newly born worm the total number of nerve cells contained in this ganglion amounts to 6241, and then it continues to increase gradually until the adult stage, in which the total number is 12829 (Chart II), the ratio between the newly born worm and the full grown one being 1:2. As seen in Table IV and Chart IV, the manner of increase of large nerve cells in this ganglion bears resemblance to that in the suboesophageal ganglion.

Besides, the number of the characteristic nerve cells is given in the same column as the total number of nerve cells. In Table II one can easily realize that the newly born worm has very few of the so-called characteristic cells, so that they are not found easily anywhere, but they increase very rapidly in number, though their size does not grow so conspicuously (Table II and Chart II). The size of cells is given as the product of the long axis by the short one.

As regards the cerebral nerves, among ten cerebral nerve trunks, I have taken only the 5th one for the calculation of nerve fibers. The result is given in Table III, while the manner of increase is illustrated by the growth curve in Chart III. As one can see from Table III and Chart III, the growth curve of the number resembles that of the ordinary segmental nerve trunks, both of them showing a relatively small rate of increase, but the growth in their size occurs more rapidly than that in their number (Compare Tables III and V). If we compare the increase in the number of nerve cells and fibers in the cerebral ganglion, we find that for the cells the ratio between the newly born worm and the adult is 1:2, while for the fibers it is 1:3.6. Generally speaking, it is reasonable to see in every case that the increase of fibers in number has taken place in a higher degree than the increase of cells. It is interesting to note further that in spite of the far different structure of the cerebral

TABLE

The increase of the number of ganglion cells in

Age (Weeks)	Cerebral ganglion		Enteric nerve Right side	Suboesophageal ganglion Right side	Head region (VIIIth segment) Right side
	Total nerve cells	Characteristic cells			
in hatching	6241±4.53	103±0.93	749±1.42	1836±2.85	481±1.52
I–II	6974±4.86	117±0.98	763±2.54	1866±2.72	554±2.16
III–IV	7543±4.63	252±1.14	762±2.83	2054±3.39	676±2.49
V–VI	8393±5.49	465±1.29	769±2.65	2247±5.56	813±2.73
VII–VIII	9461±6.03	806±2.64	774±2.43	2305±5.79	1023±3.12
IX–X	10398±6.61	1609±3.73	784±2.68	2320±6.09	1197±3.65
XI–XII	11187±6.42	2620±5.31	788±2.91	2341±6.54	1277±4.8
XIII–XIV	12388±8.73	4215±7.78	816±3.12	2490±6.27	1291±5.17
Adult	12539±8.35	5865±8.61	819±3.07	2582±6.38	1299±5.85

Chart II. The increase curves of the number of ganglion cells in the main ganglia during the growth.

No. of ganglion cells in cerebral ganglion.
No. of ganglion cells in other ganglia.

12000 2400
2000
1600
6000 1200
800
400

0   IV   VIII   XII   XVI   Weeks

----△---- Suboesophageal ganglion
-- -- ◉ -- -- Cerebral ganglion
-- -- ■ -- -- Head region ganglion
--...-□--... Clitellar region ganglion
......◨...... Prostate gland region g.
--.--●--.-- XXXth ganglion
----◐-- - LXXth ganglion
- - -▲- - - Enteric nerve thickening
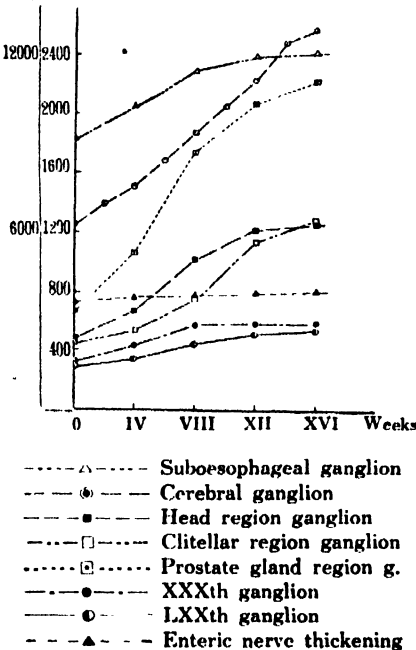
nerve trunks from that of the segmental nerve trunks, the manner of their growth curves in number is similar. However, as seen in Table V and Chart V, the growth curve for the size of the fibers in the cerebral nerves differs from that of the fibers in ordinary segmental nerves. Perhaps this great difference may indicate a characteristic nature of the cerebral nerve trunks. The measurement of the size in the nerve fibers was made as to the largest of them.

2) ENTERIC NERVES

By comparison between Fig. 23, A and D, we note at once the conspicuous appearance of the enteric nerve in the newly born worm. Upon counting the nerve cells in it, the interesting result is found, that the enteric nerve of the newly born worm contains nearly the same number of nerve

II

several ganglia during the growth.

Clitellar region (XVIth segment) Right side	Prostate gland region (XVIIIth segment) Right side	Typical segments		Tail-end ganglion (CXth segment) Right side
		(XXXth segment) Right side	(LXXth segment) Right side	
435±1.82	663±2.19	314±1.23	305±1.74	1583
468±1.98	845±2.52	354±1.29	309±1.83	1559
527±2.25	1022±3.81	427±1.89	338±1.92	1686
672±2.34	1504±4.62	540±2.04	387±2.07	1462
748±2.46	1785±5.94	569±2.34	413±2.19	1684
931±3.63	1922±7.02	568±1.92	487±2.16	1724
1191±5.61	2078±8.55	561±2.07	490±2.61	1793
1224±6.09	2176±8.22	572±2.13	572±2.64	1780
1311±7.21	2245±8.49	569±2.25	524±2.55	1358

cells as that of the adult. The number of cells in question is given in Table II, while in Chart II the manner of their increase is shown.

On the other hand, the size of these cells does not change remarkably during their growth, though only a very slight increase is proved (Table IV and Chart IV). This fact is unique in my observation through the whole nervous system of the earthworm, as I have met such a condition nowhere else. In the larvae of some insects, LOEWENTHAL ('23) and TRAGER ('36) have found that the growth of the nervous system results in the increase of the cell size, but not in that of the cell number. So far as the enteric nerves are considered my observation is different from these scientists, namely in the fact, that the size of cells in the enteric nerves does not increase during the growth of the worm. The whole enteric nerves grow in size in a very small degree, but this slight increase means to my opinion the growth of other elements, i. e. connective tissue, nerve fibers etc., but not of the nerve cells themselves. This characteristic feature of the enteric nerves may be due to their special function.

They correspond perhaps to the sympathetic nerves of the vertebrates, innervating the visceral organs. But I can not know the reason, why they show a very different manner of growth from other parts of the nervous system. To my regret, as the nerve fibers are very fine in the enteric nerves, the number of them could not be determined.

3) SUBOESOPHAGEAL GANGLION

This ganglion is generally thought to be the aggregation of three

TABLE

The increase of the number of nerve fibers in the

Age. (Weeks)	Cerebral nerves (5th trunk) Right side	Circumoesophageal commissures Right side	Head region (VIIIth segment) Right side				Clitellar region (XVIth segment) Right side			
			Double nerve		Single nerve	Neuro-pile	Double nerve		Single nerve	Neuro-pile
			Ant. t.	Post. t.			Ant. t.	Post. t.		
in Hatching	82±0.99	311±2.78	27±0.62	47±0.75	28±0.72	279±1.64	27±0.69	43±0.76	34±0.72	281±1.65
I–II	98±1.14	358±2.88	37±0.78	55±0.87	36±0.78	341±1.74	29±0.75	47±0.82	38±0.66	387±1.71
III–IV	131±1.62	431±2.94	45±0.93	65±0.96	40±0.93	421±1.71	35±0.81	59±0.98	47±0.85	439±1.75
V–VI	159±1.87	517±3.03	55±1.02	72±0.99	53±1.05	485±1.65	40±0.93	75±1.04	53±0.87	534±1.89
VII–VIII	187±2.13	635±3.72	69±1.14	108±1.44	66±0.96	559±2.04	58±1.12	93±1.16	71±0.95	628±2.19
IX–X	223±2.28	726±3.63	81±1.23	137±1.35	96±1.14	718±2.35	102±1.59	114±1.53	85±1.23	874±2.04
XI–XII	208±2.34	885±3.78	92±1.68	161±1.47	114±1.11	858±2.64	109±1.68	129±1.62	103±1.56	1082±2.23
XIII–XIV	281±2.55	1624±3.87	97±1.74	171±1.56	149±1.29	1121±3.75	113±1.83	143±1.83	108±1.62	1341±2.61
XV–XVI	288±2.64	1051±3.81	117±1.83	178±1.41	177±1.17	1196±3.39	107±1.88	140±1.95	105±1.72	1382±3.03

TABLE

The size of the largest ganglion cells in the principal

Age. (Weeks)	Cerebral ganglion		Enteric nerve		Suboesophageal ganglion		VIIIth ganglion (Head region)	
	Long axis.	Short axis.	Long axis.	Short axis.	Long axis.	Short axis.	Long axis.	Short axis.
in Hatching	26	20	16	14	26	24	25	22
I–II	27	21	16	15	32	26	30	24
III–IV	28	23	18	15	35	30	32	28
V–VI	30	24	19	17	40	32	40	30
VII–VIII	32	25	19	18	42	36	42	34
IX–X	36	27	20	18	44	38	44	38
XI XII	40	29	21	19	46	42	46	40
XIII–XIV	40	30	21	19	48	43	45	43
Adult	41	30	21	19	48	44	45	43

ganglia of the ventral cord, but it has some special ganglion cells, which are not found in the ordinary ganglia. The details on the morphology of these special cells were already spoken of in this paper. At first, I calculated the total number of cells in this ganglion, the results being

III

several nerve trunks during the growth.

Prostate gland region (XVIIIth segment) Right side				Typical segment (XXXth) Right side			
Double nerve		Single nerve	Neuro-pile	Double nerve		Single nerve	Neuro-pile
Ant. t.	Post. t.			Ant. t.	Post. t.		
28±0.65	42±0.73	45±0.84	321±2.42	20±0.55	33±0.72	19±0.63	279±2.55
31±0.72	41±0.76	46±0.86	356±2.73	25±0.62	34±0.81	26±0.79	303±2.85
34±0.84	50±0.87	48±1.29	428±2.94	32±0.84	38±0.87	32±0.88	319±3.16
45±0.67	71±1.17	49±1.38	522±3.06	34±0.99	45±1.17	37±1.02	451±3.39
76±1.18	98±1.28	55±1.53	782±3.21	38±1.08	52±1.29	45±1.18	561±3.72
97±1.35	119±1.74	66±1.71	937±3.44	46±1.14	74±1.32	54±1.23	682±3.87
118±1.71	154±2.04	119±1.83	1152±3.63	59±1.23	81±1.38	63±1.35	818±4.05
139±2.02	176±2.07	151±1.93	1482±4.26	79±1.35	90±1.44	71±1.44	979±4.41
136±1.98	188±2.13	164±1.88	1547±4.83	76±1.38	92±1.53	69±1.42	1045±4.53

IV

ganglia during the growth. (in micra.)

XVIth ganglion (Clitellum)		XVIIIth ganglion (Prostate gland)		XXXth ganglion (Typical segment)		Characteristic cells in cerebral ganglion	
Long axis.	Short axis.	Long axis.	Short axis.	Long axis.	Short axis.	Long axis.	Short axis.
24	20	23	20	26	18	14	10
27	24	28	21	27	20	15	11
30	26	30	22	28	24	16	12
32	28	32	25	32	26	17	14
37	30	34	28	34	28	18	16
41	32	38	32	36	32	20	18
46	38	42	36	40	38	21	19
46	40	44	41	44	40	22	20
46	42	46	42	46	41	22	20

given in Table II and Chart II. The manner of their increase in number resembles that in the cerebral ganglion; they grow gradually in number up to the adult stage.

These special nerve cells "1" are the greatest in size throughout the

Chart III. The increase curves of the number of nerve fibers in several parts during the growth.

No. of nerve fibers.

Chart IV. The growth curves in the size of largest ganglion cells, from the newly born to the full grown worm.

Relative units (short axis × long axis)

Chart III legend:

---•--- Circumoesophageal connectives
—··—•·— Cerebral nerve trunks
······ʌ······ VIII ganglion-neuropile
·· —▲·— XV ganglion-neuropile
···· ·■····· XVIII ganglion-neuropile
— —⊓— — Double nerve in XVIII segment
——○—— XXX ganglion-neuropile
—··—•—·— Double nerve in XXX segment

Chart IV legend:

—· ·—•·---- Cerebral ganglion
·· —▲— — Enteric nerve
·— —○——— Characteristic cells
—····ʌ·---- Suboesophageal g.
—···—⊓—·· XVIth ganglion
······⊡······ XVIIIth ganglion
—··—•—·· XXXth ganglion

nervous system of the earthworm. From Table IV and Chart IV, one can accept that the growth of their size occurs also in the similar manner as the largest cells in the cerebral ganglion, in spite of their great difference in size. To my observation, their nuclei change less in size during their growth than the nuclei of cells in other ganglia. And also, the ratio of the increase of nerve cells in number is certainly different between the suboesophageal and the cerebral ganglia, in the former the ratio between the newly born worm and the adult being 1 : 1.4, while in the latter it is 1 : 2.

4) CIRCUMPHARYNGEAL COMMISSURES

As a representative of nerves which leave the suboesophageal ganglion,

TABLE V

The size of nerve fibers in the principal parts
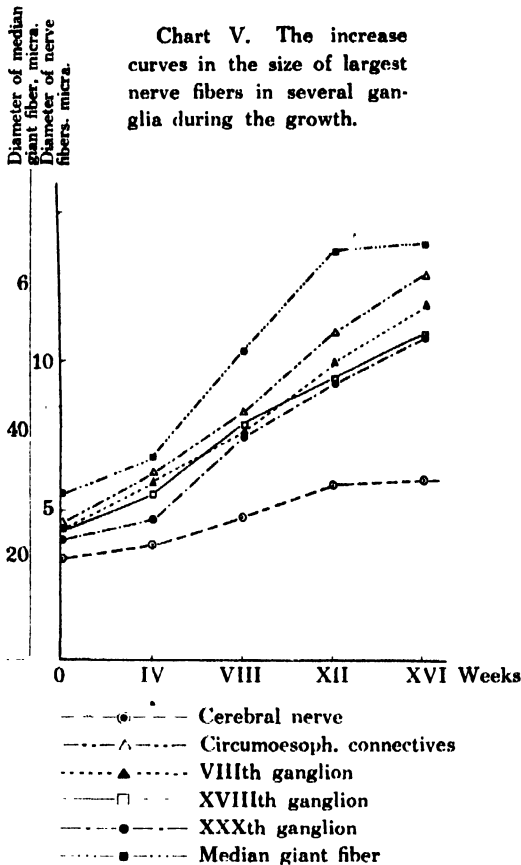during the growth.

(in diameter in micra)

Age. (Weeks)	Cerebral nerves (5th nerve)	Circum-pharyngeal connective	VIIIth ganglion neuropile	XVIth ganglion neuropile	XVIIIth ganglion neuropile	XXXth ganglion neuropile	XXXth ganglion median giant fiber
in Hatching	3.4	4.5	4.3	4.3	4.1	4	28
I-II	3.5	5.8	5.6	5	4.8	4.6	30
III-IV	3.8	6.2	6	5.6	5	4.8	34
V-VI	4.4	7.5	7.2	7.2	7	6.5	47
VII-VIII	4.8	8	7.6	8.3	8	7.8	52
IX-X	5.5	10	9.6	8.6	8.5	8.1	51
XI-XII	6	11	10	10	9.6	9.4	70
XIII-XIV	6	12.5	12	11	10	10	70
Adult	6	13	12	11	11	11	70

the circumpharyngeal connectives, which is united with the cerebral ganglion, was observed. The structure of this connective is essentially similar to that of the neuropile in the ventral cord, as mentioned already in the morphological chapter. The results of the enumeration were given in Table III, while the increases of the number and size are represented in curves in Charts III and V. The increase of number takes place in a similar manner to that in the neuropile of the ganglia of the ventral cord. From Table V and Chart V, one can also see that the increase of size shows the same feature as in the neuropile of the ventral cord.

5) GANGLION OF THE HEAD REGION

In my previous paper I stated that the cell-number in the ganglion of the head region is much greater than in the typical segment, owing to the structural complexity, because it has various reproductive and other organs.

At present I intend to study how the cell-number increases in the ganglion of the 8th segment during the growth. Table II indicates my results on the cell-number, Table III presents the amount of nerve fibers belonging to this ganglion at every growth period, while Charts II and III show their growth curves. From these tables and charts it is clear that already in the newly born worm the ganglion in question has more cells than any other ganglion, except the cerebral and suboesophageal

Chart V. The increase curves in the size of largest nerve fibers in several ganglia during the growth.

Diameter of median giant fiber, micra.
Diameter of nerve fibers, micra.

— ·· —◉·— — — Cerebral nerve
—··—∧—···— Circumoesoph. connectives
······▲······ VIIIth ganglion
———⊓ · · XVIIIth ganglion
—·—·—●—·—· XXXth ganglion
······—■—······ Median giant fiber

ganglia. The rate of increase is also quite different from that in other ganglia; it shows a characteristic growth curve. In a worm ten weeks after birth the cells reach the full number, i. e. equal to the number in the full grown worms. While the number of nerve cells generally continues to increase in the parts with reproductive organs till the rife period, one meets in the concerned ganglion a different condition, inasmuch that the increase ceases relatively early, although it contains the spermatheca and its opening. Therefore it seems to me that the spermatheca influences the number of nerve cells in a different manner than the prostate gland in my previous results.

As to the number of nerve fibers it is in the neuropile of this ganglion in the newly born worms almost equal to that in the typical segment, while in the peripheral nerve trunk it is somewhat larger than in the ordinary segment. In the adults the number of nerve fibers becomes much greater both in the neuropile and in the peripheral nerve trunk, and especially in the latter. As Chart III indicates, the growth curve showing the increase of nerve fibers in the neuropile is on the whole similar to that of the typical segment, i. e. the 30th segment, while the peripheral nerve trunk differs remarkably in this relation from the condition in the 30th segment.

As the next problem I studied the change of cell-size during the growth of worms. For this purpose the greatest ganglion cells " sM$_3$ " were chosen as the representative ones. They are characteristic for this

ganglion, and situated in the middle portion of the line combining the bases of the single nerves of both sides, as I have already mentioned about them in the morphological chapter. Measurements were taken of these cells in several individuals at each stage of growth from the newly born to the adult worms. Table IV and Chart IV indicate my results. From the data I conclude that the cell-size as well as its growth curve resembles in a high degree the relation of nerve cells in the suboesophageal ganglion.

On the other hand, the growth rate in the size of the nerve fibers is very similar to that in the typical segment, as seen in Table V and Chart V.

In short, the influence of the reproductive organs upon the number of nerve cells in the ganglia in this region is relatively small. The general feature in the typical segments is also met with in this ganglion.

6) Ganglion of the clitellum region (XVI segment)

As the clitellum has the most intimate relation with the reproductive organs, it is interesting to know how the nerve cells in the ganglion of this part of the body change in number and size during growth. As an example the ganglion in the 16th segment was examined. In the newly born worms the number of nerve cells in this ganglion is somewhat greater than in the typical segment (XXX segment), but it is less than in the ganglion of the head region. But in the adult worms the number in question remarkably surpasses that of the ganglion of the head region amounting to more than twice as many as in the typical segment. The increase of the nerve cells continues gradually in the adult worms, clearly being much influenced by the growth of reproductive organs. On the other hand, in the typical segment the full number is already attained in the 5th week.

Contrary to the nerve cells, which are fewer in newly born worms in the clitellum region than in the head, the number of nerve fibers passing through the neuropile is larger than in the ganglion of the head region, as Table III shows. It is perhaps because the nerve fibers descending from the more anterior portion are fewer in the head than in the clitellum region. In the segmental nerve trunks there is no great difference between both regions, the number resembling that in the typical segment (Table III). Now, the manner of the increase of cells in the clitellar region, as seen in Chart II, indicates similar feature as in the XVIII segment (with

the prostate gland), while the fibers show the intermediate relation between the typical and the XVIII segment (Chart III).

The increase of the fiber-size in the neuropile occurs in the ganglion in question, as Chart V indicates, in a similar manner as in that of the XVIII segment with the prostate gland. The increase rate of the fibers in the neuropile between the newly born and adult worms is 1 : 3, showing the ratio very similar to that in the ganglion of region of the prostate gland. In the segmental nerve trunks the rate of increase is not so similar between both of these regions as in the neuropile.

The growth of the cell-size was studied in the same sort of nerve cells as treated in the foregoing chapter, i. e. the VIII segment. The ratio of increase is considerably similar between the VIII and XVIII segments, though the increase takes place a little later in the latter. This fact seems to accord with the relation that the development of the body of the worm proceeds in the direction of anterior to posterior. Also the size of the nerve fibers increases in nearly the same manner as that of the nerve cells. I think it is a natural phenomenon, which Table IV and Chart IV show. Briefly, the ganglion in the clitellum region differs much in its development from that of the typical segment; it is intimately related with the growth of the reproductive organs.

7) GANGLION OF THE PROSTATE GLAND REGION (XVIII SEGMENT)

While studying the number of nerve cells in the adult worms, I was surprised that the ganglion of the prostate gland region has the greatest number of them, next to the suboesephageal ganglion. I am much interested to know whether this great number is also seen in the embryos or in the newly born worms. At present I ascertained that the number in this ganglion amounts in the newly born worms already to twice as many as that in the typical segment and afterwards increases rapidly.

At the adult stage the number of the cells in this ganglion attains to 4.3 times as many as that in the typical segment. The increase of nerve fibers proceeds more remarkably, reaching 5 times as many as in the adult worm (Table IV), while in the segmental nerves the increase is not so conspicuous, amounting to about 3 times as many.

The special manner of the increase of cell-number is shown in Chart II; it differs from the relation in other ganglia. This very rapid increase of the cell-number in this ganglion clearly depends upon the development of the prostate gland, while the most rapid increase of the number of

nerve fibers is indicated in Table III and Chart III.

The growth rate of the cell-size "sM$_2$", which was also studied in the same kind of cells as in the other ganglia, shows no particular relation in comparison with the head and clitellum regions (Table IV and Chart IV). The same is true about the growth of fiber-size in the ganglion of the prostate gland region (Table V and Chart V). It is not specially different from the relation in the typical segment.

8) Ganglia of the typical segments (XXX and LXX)

By the typical segments I mean the greater part of the earthworm, that ranges in the present species from about the 20th to 105th segment excluding the tail end. In them the structure varies in the least degree and is, therefore, the most convenient for comparison of the growth throughout the whole body. For this reason they have often been used for comparison in this work, when considering the other parts of the worm.

The increase of the number of nerve cells in the typical segments differs from that in the ganglia of the ventral cord mentioned already. For instance, in the XXX segment the full number is attained in 5–6 weeks, and in the LXX segment at the latest in 7 weeks after hatching (Table II). In Chart II it is clearly shown that the growth curve of the cell-number ascends very slowly; in other words, the growth rate is low. If we compare the anterior (XXX) and posterior (LXX) segments, the growth in the former takes place more rapidly, reaching the full number in a shorter time than in the latter. This fact means that the growth of the earthworm proceeds from the fore part gradually to the hind part.

The next problem concerns the increase in the number of nerve fibers. In the newly born worms the number of nerve fibers in the neuropile of the typical segment is nearly equal to that of nerve cells in the concerned ganglia, while in the adult stage the former surpass the latter almost twice. The same condition was also met with in the neuropile of the head region, as Chart III indicates this relation. While the cell-number already reaches its maximum in 5–6 weeks after hatching, the nerve fibers continue to increase in number till in the adult stage. I take it to be quite reasonable that the increase of cell-number occurs earlier than that of nerve fibers.

As for the segmental nerves, the number of nerve fibers in the adult amounts to twice as many in comparison to those in the newly born

worms, similar to the neuropile, but as Table III shows, the full number is attained earlier in the segmental nerves than in the neuropile.

The cell-size "sM₃", which is equal in the newly born worms between the ganglia of both (anterior and posterior) segments, increases relatively slowly and reaches the full size in 12–13 weeks after hatching in the XXX as well as in the LXX segment. On the other hand, the increase of fiber dimensions measured in the neuropile takes place in nearly the same manner between both ganglia of the ventral cord (Table V, Chart V), though in the anterior ganglion the nerve fibers seem to grow somewhat earlier, as the fiber-size in it, compared in the same stage, is generally a little larger than in the posterior ganglion. To compare with this relation, the size of the median giant fiber was measured. It does not show a much different growth rate than the nerve fibers in the neuropile (Table V and Chart V).

9) GANGLION OF THE TAIL END

According to my observation, after the birth of the earthworm only 7 or 8 segments seem to be added successively to the tail end, and this increase of the segments, namely of the ganglia, occurs during about 12 weeks after hatching.

The number of nerve cells is very variable in the ganglion of the tail end; there are quite many in some individuals, in others very few. So I could not get any definite result about this ganglion, except that the number in it in the newly born worm is three times as large as in the typical segment. The data about the growth were very inconstant in this ganglion, differing remarkably from the relation in other ganglia.

10) INCREASE OF MUSCLE FIBERS IN THE EARTHWORM DURING THE GROWTH

From the functional standpoint the muscle fibers have the most intimate relation with the nervous system, and consequently they have been very often treated in the studies about the latter. For example, SMALLWOOD ('26) took into account the numerical relation between muscle fibers and nervous elements in certain segments, when he insisted upon the presence of the subepidermal network. Recently PROSSER ('35) studied responses of the earthworm by stimulating some areas of the epidermis, giving special attention to the relation between sense organs, ganglion cells, and muscle fibers. Their opinions are, however, based partly upon

the numerical data reported by other authors, and, as the materials and the parts of the body observed are not always identical between the authors, a renewal of the study of the numerical relation between nerve cells and muscle fibers would be necessary.

So I intend to calculate the muscle fibers and sense organs in the definite segments treated in the previous chapters in order to compare them with the number of nervous elements and to know the correlation between them. In *Pheretima communissima*, the muscle fibers of the body wall are arranged in two layers as in other Oligochaeta, an outer circular and an inner longitudinal.

My own experiments and observations were undertaken in the following way. The worms were studied at four stages of development from hatching to the adult, namely newly born, 5 weeks, 10 weeks and 15 weeks after birth. Of each stage 2 or 3 specimens which belong to the standard type, were examined. It was very difficult to calculate exactly the cells of the muscle layers and epidermis, for they are very great in number and not fitted for observation, otherwise than in the case of the ganglion cells; so I was obliged to estimate only an approximate number of them. For this purpose I used the same preparations which were studied in respect to nerve cells and fibers; they were treated by HEIDENHAIN's haematoxylin and cross-sectioned. The calculation was made at the VIII segment (head region) and XXX segment (typical segment), as seen in Table VI, and the number of muscle fibers was determined by

TABLE VI

The increase of muscle fibers (largest f.) during the growth.

Age (Weeks)	Number				Size (in mm.)			
	Longitudinal muscles		Circular muscles		Longitudinal muscle f. in mm.		Circular muscle f. in mm.	
	VIIIth segment	XXXth segment	VIIIth segment	XXXth segment	Length	Diameter	Length	Diameter
Newly-born	21600	5950	8928	3050	0.8	0.008	0.5	0.01
V	44260	25800	26320	10800	1	0.018	0.8	0.019
X	62750	34030	30528	13320	1.8	0.02	1.1	0.021
XV	72200	42864	31600	16992	3.5	0.021	1.3	0.022

counting their nuclei. For small worms every 3rd and for larger ones every 5th preparate was observed and from the results the total number in one segment estimated. Table IX indicates the data obtained. Already
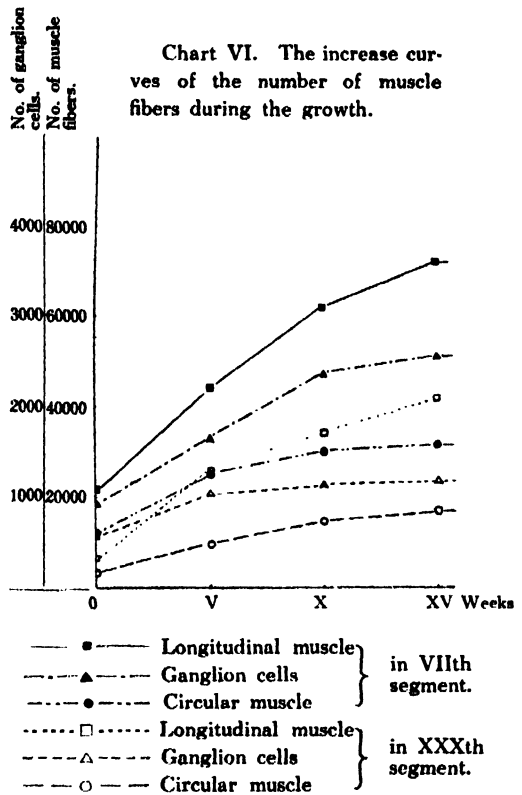
in the newly born worm the number of longitudinal and circular muscle fibers altogether amounts to about 15,000, that is 35 times as many as the number of nerve cells in the ganglion of the ventral cord. If we compare the number of muscle fibers calculated by SMALLWOOD ('25) in the adult worm of *Eisenia foetida* with my result in *Pheretima communissima*, the latter surpasses the former nearly by 10 times. But, according to my calculation in *Eisenia foetida*, the longitudinal muscle fibers amount to about 9,000 instead of about 10,000 estimated by SMALLWOOD, while the circular ones show the number of 3,600 in a typical segment. There were nearly the same results in both cases. The adult worm of *Pheretima communissima* has to my observation about 100,000 longitudinal muscle fibers, i. e. 10 times as many as the corresponding number estimated by SMALLWOOD and in my observation of the adult *Eisenia*.

It is an especially interesting fact that the ratio between the muscle fibers of a segment and the nerve cells in the ganglion (35 : 1) does not change remarkably during the growth from the newly born worm to the adult stage. Comparing the number of efferent fibers in the segmental nerves in the corresponding segment with that of muscle fibers, the former is about 1,000 altogether in 3 pairs of segmental nerves, while the latter, longitudinal and circular muscles being counted, is about 100,000, amounting therefore to nearly 100 times as many as the efferent fibers. The ratio between the muscle and efferent nerve fibers could not be seen exactly. The fact that the nerve fibers are remarkably fewer in number in comparison with the muscle fibers is to be explained by the two assumptions that an efferent fiber gives off many branches to a number of muscle fibers and that moreover the intermuscular nerve cells are present in a greater degree than we have expected, issuing many branches to muscle fibers, In Chart VI the rate of increase in the number of muscle fibers is indicated and for the sake of comparison a curve relating to nerve cells is added. According to the figures the longitudinal muscle fibers and nerve cells increase in number nearly in the same manner, while the rate of increase of the circular muscle fibers is relatively small. If we compare the number between the longitudinal and circular muscles, the latter is comparatively less in. the newly born worms than in the adult and at the adult stage the number of circular muscle fibers is about one half that of the longitudinal muscle fibers. The last mentioned ratio agrees approximately with SMALLWOOD's estimation in *Eisenia foetida.* Moreover in *Branchiobdella,* a very small Oligochaeta, in each half of a segment the number of circular muscles is said to be 25, while that of

the longitudinal muscles is 44 (SCHMIDT, '03); the ratio between them coincides with the above mentioned result.

As the next problem I wish to study the change in form and size of muscle fibers during the growth. By treating them with 32% nitric acid and the saturated solution of potassium chloride the muscle fibers were isolated. According to my observation, the circular muscle fibers are of the so-called nematoidal shape (Fig. 36), while the longitudinal ones are very long and of the ribbon-like column shape. The nuclei almost always lie in the middle of the fiber. The form of muscle fibers in cross section, which is drawn as to the newly born and adult worms in Fig. 36, is very similar to that of *Lumbricus*, etc.

Chart VI. The increase curves of the number of muscle fibers during the growth.

	Longitudinal muscle	in VIIth segment.
	Ganglion cells	
	Circular muscle	
	Longitudinal muscle	in XXXth segment.
	Ganglion cells	
	Circular muscle	

The change in size of the muscle fibers is shown in Table VI and Chart VII. But as I have measured them after fixation, the natural size must be somewhat larger than the data indicated in the table. By fixation the muscle fiber seems to become shorter about to 2/3 of the natural size. The longest of the longitudinal muscles is 3 mm, while that of the circular is 1.3 mm. According to STEPHENSON ('30), in small Oligochaeta, for example *Tubefix, Limnodrilus lumbriculus*, the longest muscle fiber measures only 55 micra (STEPHENSON, p. 55), while in *Lumbricus terrestris* this length is said to be 4.3 mm (SCHNEIDER). My own result bears resemblance of the length of the muscle fibers in *Lumbricus*. In the newly born worms the longitudinal muscles are smaller in length than 1/3 of the corresponding muscles in the adult worms, while the circular ones are between 1/3 or 1/2 of the wholly developed muscles. Also in respect to the diameter the longitudinal muscle fiber is far greater in the adult worm than in

the newly born (about 4 : 1) ; the ratio is for the circular muscle fibers
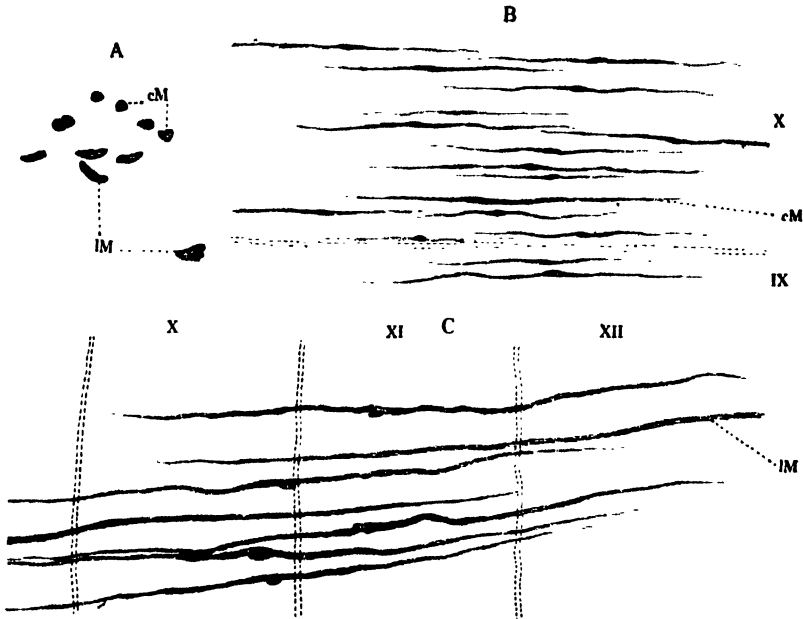about 2 : 1.   In addition I intend to describe the distribution of muscle

Fig. 36.   The muscle fibers. A) The circular and longitudinal muscle fibers
in the cross-section in the 8 weeks old worm; ×350.   B) The arrangement of
the circular muscle fibers in the Xth segment of the newly born worm by the
surface view; ×100.   C) The arrangement of the longitudinal muscle fibers in the
Xth, XIth, and XIIth segments of the newly born worm by the surface view; ×100.
cM = circular muscle fibers, lM = longitudinal muscle fibers, X–XII = no. of segment.

fibers, which was clearly ascertained in some preparates treated by the
intravitam method with methylene blue.   A circular muscle fiber is
restricted in one segment; it does not reach the next segment, while
nearly all longitudinal muscle fibers extend themselves over 2–4 segments.
These facts are shown in Fig. 36.   As the similar relations are also seen
in the newly born worms, the growth of the muscle fibers seems to go
nearly parallel with that of the worm body itself.   To illustrate these
relations Chart VII was made, in which the increase in length should
be compared between muscle fibers and the worm body.   The curves are
very similar between them.

11) INCREASE OF EPIDERMAL CELLS DURING THE GROWTH

Of all the organs of the earthworm the body wall is the most appropriate to study concerning the growth rate in comparison with that of the ganglion cells and the muscle fibers. The body wall contains gland cells, supporting cells, basal cells, and setae, and I intend to observe the increase of the number and the size of these elements during the growth. For this purpose I used the same materials which were studied in the observation of nerve cells and muscle fibers, namely the cross-sectioned preparates treated with haematoxylin and the other preparates stained vitally with methylene blue. For the comparison of cell-size only the preparates made by the same technique were used and only the epidermal cells in the middle

Chart VII. The growth curves in the size of the muscle fibers during the growth.

- – – c – – – Length of longitudinal m.
—·––·(?)—·– Length of circular m.
—·– –•– · – Diameter of circular m.
······ ■ ······ Diameter of longitudinal m.

portion of the VIII segment were observed. Here again the whole growth period was divided in 4 stages from the newly born to the full grown worm, as in the study of muscle fibers. As the VIII segment is situated in the head region the number of epidermal cells in it is generally larger than in the typical segment. For the enumeration of the epidermal cells I counted every other 3rd or 5th section; as Table VII and Chart VIII show, the number of epidermal cells, all sorts of them taken together, is larger than that of the muscle fibers and also than that of the nerve cells, while the change of the size of epidermal cells during the growth is quite different from other sorts of cells, as they become greater nearly exclusively in length, but almost do not grow at all in width.

Moreover, the size of their nuclei remains almost unchanged from the newly born to the full grown stage, except that some of them are altered in form owing to the pressure from the neighbouring cells. In the larva of insecta observed by TRAGER ('36) the nuclei in the hypodermal
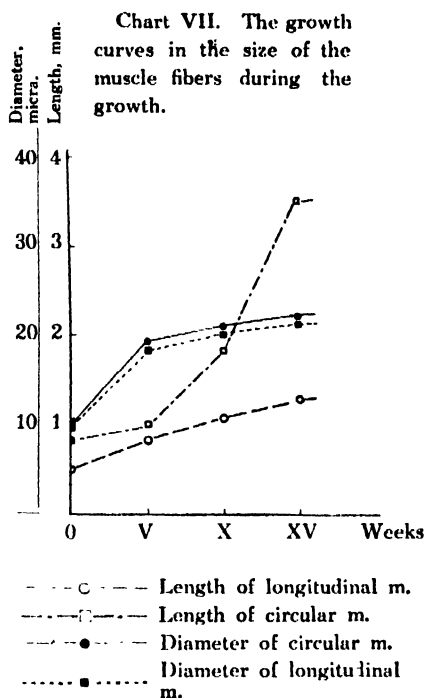
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TABLE VII

The increase of epidermal cells in VIIIth segment
during the growth.

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Age (Weeks)	Number				Size (in micra)						
	Gland cells	Supporting cells	Basal cells	Sensory cells	Gland cells	Supporting cells	Sensory cells	Setae		Basal cells	
								Length	Diameter	Length	Width
Newly born	5649	10350	3952	2316	17	20	25	69	10	7.5	8.5
V	20700	60900	24000	11240	25	31	38	140	12	10.0	8.5
X	36820	104720	54180	21280	37.5	39	45	300	24	11.6	10.6
XV	70080	203040	109680	26600	43.7	43.7	50.6	363	32	13.0	10.6

cells are said to increase in size as much as 4.5 times during the growth. But in my materials, as Table VIII shows, the nuclei of nerve cells and muscle fibers increase in a certain degree, but not so remarkably.

Generally speaking, among the epidermal cells the supporting cells show the great increase in number (1 : 20), while the basal cells show the least (1 : 2.8). On the other hand, the gland cells increase in size in the highest degree (1 : 2.6), while the growth rate of other sorts of cells is about 1 : 2.

i) Gland cells.

As in *Lumbricus* and *Eisenia* we find in the present species two kinds of gland cells; one of them secretes mucus, while the other produces an albuminous substance. The gland cells are the largest among all of the epidermal cells; their shape is elliptic on the cross-sectioned preparates and round on the surface view preparates. About the distribution of gland cells in the epidermis of *Pheretima* the following facts are recognized; first, that they are almost absent in the middle portion of a segment, where the setae exist in a row, making the so-called setal line, and where many sense organs are present, and second, that no gland cells are seen in the part between two neighbouring segments.

During the growth period the mucus gland cells increase only in length, while their breadth hardly becomes larger at all. Therefore I recorded only the growth of their length. The size of their nuclei does not change, as is also the case in the other sorts of epidermal cells. These results contradict TRAGER's ('36) view, that the size of nuclei in the hypodermal cells increases in the larva of insecta during the growth. On the other hand the number of gland cells shows the similar ratio of increase as

that of supporting cells (Chart VIII).

ii) Supporting cells.

The supporting cells constitute the greatest part of the epidermis; their form is cylindrical, similar to those of *Lumbricus* and *Eisenia*, the outer surface covered with cuticule is straight, and the opposite side, becoming remarkably smaller in breadth, is attached to the base

Chart VIII. The increase curves of number of epidermal cells in the VIIIth segment during the growth.
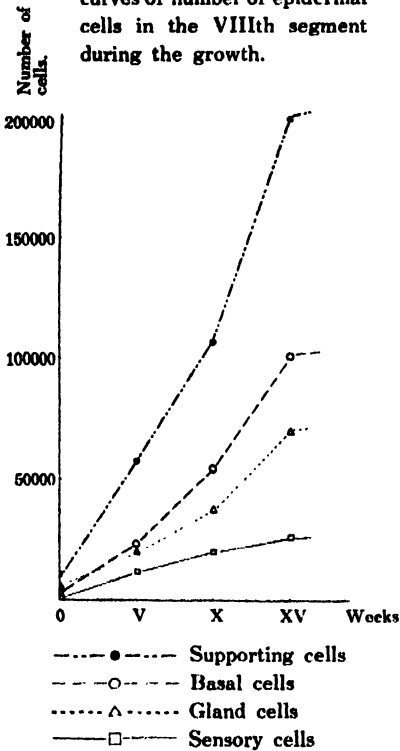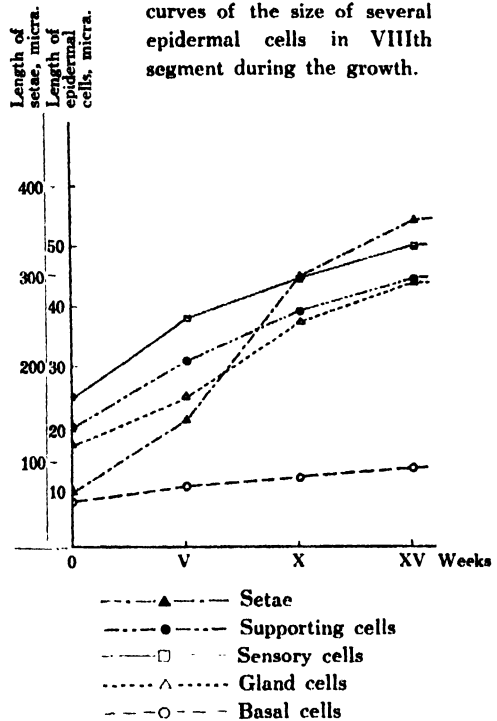
Chart IX. The increase curves of the size of several epidermal cells in VIIIth segment during the growth.

— ·· — •——·· — Supporting cells
— — —○— — — Basal cells
······ ∆ ······ Gland cells
———□——— Sensory cells

— ·· — ∆ — ·· — Setae
— ··· — •———·· Supporting cells
— ·· ———□ ·· — Sensory cells
······ ∆ ······ Gland cells
— — —○— — — Basal cells

membrane. The nucleus which contains much chromatin granula and can be easily discerned from other epidermal cells by this characteristic, is situated at about the middle of the cell.

The number of the supporting cells increases during the growth, as Table VII and Chart VIII indicate, very remarkably among all sorts

of epidermal cells, in the full grown stage amounting to 20 times as many as the number of them in the newly born worm. On the other hand, the increase of the cell-size takes place, as seen in Table VII and Chart IX, almost exclusively in length, but not in width, as the same relation was proved about the gland cells. Moreover the nucleus, which is round in shape in the newly born stage, changes gradually with the development to an elliptic form owing to the pressure from the neighbouring cells. The size of nucleus seems, however, to remain almost unchanged.

iii) Basal cells.

These cells lie in direct attachment to the base membrane and are said to be changeable in some other sorts of epidermal cells. Their form is quite different from other cells in the epidermis ; in shape as well as in size they are very variable.

The number of these basal cells increases during the growth in the same manner as that of supporting cells and gland cells, reaching in the full grown worm the number of about 30 times as many as compared with them in the newly born stage. The size of the concerning cells was measured from the largest of them. From these results it is easily known that the basal cells increase in length only in a small degree, compared to other epidermal cells ; the length of the basal cells in the adult worms is twice as large as that in the newly born. Their width also increases only a little during the growth period. Their increase takes place more in number than in the dimensions of each cell. The size of their nuclei which are the smallest among the epidermal cells, does not change remarkably, resembling in this relation the nuclei of other epidermal cells.

iv) Sensory nerve cells.

Sensory nerve cells in the epidermis may be classified generally in two sorts, one of them is represented by the cells, existing solitarily between other epidermal cells, while the other sort comprises those, which constitute the sense organs. Both of them I have observed in the sectioned preparates, stained with haematoxylin, as well as by the surface view after vital staining with methylene blue. In the newly born worms the sense organs and the sensory cells, which constitute the organs, are few in number. The ratio of their increase is very low, differing in this relation from other epidermal cells. In other words, the sensory nerve cells exist in the newly born worms in a relatively large number, compared with other epidermal cells. This fact agrees with the assumption, that the

nervous elements are developed generally earlier than other sorts of cells. According to TRAGER, the nerve cells in the larva of insecta show also a very low ratio of increase especially when compared with other somatic cells.

The length of the sensory cells in the sense organs increases in a higher degree than that of other epidermal cells, as they are bent in the shape of a bud and so comparatively are the largest in length. Table VIII and Chart IX show the results of my measurements of the size of the cells, which constitute the sense organs. The diameter of their nuclei does not increase after hatching, as is also the case in other cells.

v) Setae.

In *Pheretima communissima* the typical segment has generally 60 setae, while in segment VIII 62 of them are calculated. The number of them does not increase after hatching; their size only becomes greater. Table VII shows the data of the growth of setae in size and number. A seta is said according to BAIIL ('25), in the full grown *Ph. hosthuma* to measure 0.26 mm in length and 0.032 mm in diameter, while the corresponding numbers in the present object are 0.36 mm and 0.032 mm respectively. The increase-ratios of diameter and length of the setae during the growth period are different from the relations in other epidermal cells ; they increase as much as 6 times in length, and 3 times in diameter, compared with the newly born stage.

12) SUMMARY ABOUT THE NUMBER OF GANGLION CELLS, NERVE FIBERS AND OTHER CELLS CALCULATED DURING THE GROWTH

In order to show clearly the increase ratios of various elements of the earthworm during the growth, I prepared Table VIII in which the whole time from hatching to the full grown worm was divided in four stages, and the size and number enumerated at each stage are compared with the corresponding data measured in the newly born worms.

Of all the elements treated by me in the present paper, the basal cells in the epidermis show the greatest increase, amounting to about 28 times as many as the number in the newly born worm. Generally speaking, the increase ratio of the number of epidermal cells is much greater than that of the nerve cells and the muscle fibers. The increase of muscle fibers is also tolerably great, especially that of the longitudinal muscle fibers is conspicuous. On the other hand, the increase of ganglion cells occurs in the slightest degree, attaining the number only

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TABLE VIII.

The increase ratios during the growing period on bases
of data at hatching as 1.

Elements	Age (Weeks)	in Hatching	V	X	XV
Body	Weight	1	10.2	68	208
	Length	1	2	3.6	6.1
	Diameter	1	2	3	4.4
Ganglion cells	Number	1	1.4	2.4	2.7
	Length	1	1.1	1.4	1.8
	Width	1	1.4	1.8	2.3
	Nucleus	1	1	1.1	1.1
Muscle fibers (Circular)	Number	1	3.5	4.3	5.5
	Length	1	1.6	2.2	2.6
	Width	1	1.9	2.1	2.2
	Nucleus	1	1	1	1
Muscle fibers (Longitudinal)	Number	1	4.3	5.7	7.2
	Length	1	1.2	2.3	4.4
	Width	1	2.3	2.5	2.6
	Nucleus	1	1	1	1
Gland cells	Number	1	3.6	6.5	12.2
	Length	1	1.3	2.3	2.6
	Width	1	1	1	1
	Nucleus	1	1	1	1
Supporting cells	Number	1	5.9	10.1	20
	Length	1	1.5	1.9	2.2
	Width	1	1	1	1
	Nucleus	1	1	1	1
Basal cells	Number	1	6.1	13.7	27.5
	Length	1	1.3	1.5	1.7
	Width	1	1	1	1
	Nucleus	1	1	1	1
Sensory cells	Number	1	4.8	9.1	11.5
	Length	1	1.5	1.9	2.2
	Width	1	1	1	1
	Nucleus	1	1	1	1
Setae	Length	1	2.3	5	6.1
	Width	1	1.2	2.4	3.2

about twice as many as those seen in the newly born stage. The least remarkable increase of nerve cells after hatching seems to be a general feature throughout the animal kingdom, for in mammalia the nerve cells are not said to grow in number after birth.

Contrary to this, the increase of cell-size takes place to a far greater degree in the nerve cells than in the epidermal cells, which become larger only in length, and the basal cells, characterized by the greatest increase in number, show the slightest growth in size. Certain of the largest ganglion cells, whose number increases only a very little, reach the size several times larger than in the newly born stage. These results are not coincident with the remarks of TRAGER ('35) that the increase in the size of ganglion cells in the larva of insecta is much smaller than that of other somatic cells.

VI. GENERAL CONSIDERATIONS

From the foregoing observations about the histology and numerical relation of the nervous system, I wish, in the present chapter, by summarizing the data to consider several points which may be significant in the physiology of the earthworm. In addition, I should like here to consider the nervous elements of some other invertebrates, which are phylogenetically near to the earthworm.

i) As to the ganglia of the ventral nerve cord, it may be remarked that a larger nerve cell innervates numerous muscle fibers, giving off many short branches during the course. This fact corresponds well to the numerical relation between the muscle and nerve fibers; namely the number of the efferent nerve fibers contained in the segmental nerve trunks is remarkably smaller than that of the muscle fibers in the segments. On the other hand, from the existence of numerous large interganglionic cells, which send their processes to two or three adjacent ganglia, it is very easy to understand that the number of nerve fibers passing through the neuropile of the interganglion is two times larger than that of the nerve cells contained in the typical ganglion of the ventral cord. According to the previous investigators, the large nerve cells, which resemble closely the above mentioned cells of the earthworm in their position, size, processes, staining reaction and shape, are also present in the ventral nerve cord of Polychaeta, Hirudinea, Crustacea and Insecta, while in Nemertinea (Ascaris) we meet the motor neurons, which are far different from those of the higher invertebrates related above.

In the ganglia especially of the head and the prostate gland regions of the earthworm there are found abundant small unipolar nerve cells, each issuing a short process, which does not leave the ganglion itself. This fact seems to explain the numerical relation, that in spite of numerous nerve cells existing in the ganglion of the prostate gland the number of nerve fibers calculated in the concerning segment is relatively few.

ii) The suboesophageal ganglion contains three kinds of nerve cells; the first of them is of the connective nature to the cerebral ganglion, while the second is the special cells, whose processes are confined in this ganglion, and the third is represented by the usual cells which are also found in the typical ganglia of the ventral cord.

To my observation, a certain ganglion cells belonging to the first type give off their processes through the cerebral ganglion into the cerebral nerve trunks, and some other send their axons through the same ganglion into the enteric nerve thickening. In addition, it may be noteworthy, that especially in the suboesophageal ganglion the nerve cells reach the full number in the 10 weeks old worm, while they reach it earlier in the ganglia of the typical segments. In many other invertebrates, except Nemertinea, the suboesophageal ganglion seems to show the same structure as in the earthworm, for this ganglion is only a specialized portion of the ventral nerve cord, and the latter itself is composed histologically in a similar manner between a large number of invertebrates, as already mentioned.

iii) In the dorsal layer of the cerebral ganglion we find in the earthworm the characteristic small nerve cells, which differ greatly from the other sorts of nerve cells and do not give off their axons outside of this ganglion, and also a large number of the ordinary ganglion cells which are also present in the ventral ganglia. The former must perform a function special for the cerebral ganglion. The characteristic small cells in question correspond, to my opinion, to the " kleine Zellen des Gehirns " described by KRAWANY in *Eisenia foetida*. The brain of *Nereis* seems to have also the small ganglion cells similar to these nerve cells in the earthworm; they are called in *Nereis* by HANSTRÖM ('26) as the commissural small nerve cells. *Hirudo* has also such cells, which are but smaller and more compactly arranged in the cortical layer of the brain than in the earthworm (HAMAKER '98). According to HANSTRÖM, the same sort of cells is also present in the brains of Crustacea ('24), Mollusca ('26) and Insecta ('28). From these facts it may be clear, that these small nerve cells characteristic for the brain are widely seen in the

higher invertebrates. The cerebral nerve trunks contain nerve fibers coming from the unipolar small cells in both lateral sides of the cerebral ganglion; but the numerical relation between the nerve fibers and these small cells could not be clearly ascertained in the present work. As the cerebral nerve trunks are distributed in the head regions, also the small cells in question must partake in an important nervous function of the head. Moreover the brain of the earthworm has the pseudounipolar ganglion cells in its ventral part; they also must have some function proper for the brain. Similar cells have been extensively found in many higher invertebrata, i. e. Nereis, Hirudo, Mollusca, Crustacea and Insecta.

The nerve cells in the cerebral ganglion continue to increase their number during the whole period of growth from the newly born to the full grown worm.

iv) The enteric nerve shows an intimate relation with the cerebral and suboesophageal ganglia, and may be considered as a specialized ganglion for the visceral organs.

In the whole nervous system, the number of the ganglion cells in the enteric nervous system reaches its full number already at the earliest stage, i. e. in the newly born worm.

v) As to the intermuscular nerve cells, I have discerned three types of them in *Pheretima communissima*, according to their position, shape, size, nuclear structure, and staining reaction. One type is represented by the bipolar cells, which are found in diffuse condition in the baseepiment, and resemble closely the sensory cells in the epidermis, while two other types, quite different from the sensory cells, bear resemblance rather to the motor or associative neurons situated within the ventral nerve cord. The processes of the former (sensory cell type) extend into the baseepiment or in the circular muscle layer, but do not enter the central nervous system; these features let us assume that they have an associative rather than a pure receptor function. On the other hand, the latter (motor cell type) send one process into the ventral nerve cord, and the other to the muscle layers or into the epidermis.

The number of the intermuscular nerve cells calculated by me corre-sponds exactly to the estimation reported by ZYENG ('30). From the evolutionary standpoint, the intermuscular nerve cells in the segmental nerve trunks and in the circumoesophageal connective seem to be the residue of the motor cells of the primitive nerve net, which have not yet been incorporated into the ventral nerve cord. In addition, it is inte-resting that the intermuscular nerve cells, which are seen along the single

nerve trunk, send their processes partially into the epidermis.

In Coelenterata the nerve-net system seems to resemble from its distribution the intermuscular nerve cells in the baseepiment of the earthworm, while the bipolar motor cells reported in the nervous layer of Echinoderms (R. MEYER, '06) may be similar to the intermuscular nerve cells located in the interspace between the circular and longitudinal muscle layers in the earthworm.

Meanwhile, the nerve cells in Nemertinea seem to have the most intimate relation with the intermuscular nerve cells of the earthworm, as the bipolar associative cells, as reported in *Ascaris* by GOLDSCHMIDT ('09), being arranged in the ring form, and connected with the central and sensory nerves, resemble closely the intermuscular nerve cells situated in the space between both muscle layers of the earthworm; especially in the embryo of the latter the intermuscular cells in question look as if they were the same as the associative nerve cells in Nemertinea.

If we consider further the nerve cells in *Nereis,* much resemblances exist in their structure, staining reaction, and distribution between the earthworm and *Nereis;* the sensory cells in the hypodermis described by HAMAKER in *Nereis* seem to correspond to our sensory type of the intermuscular cells, which are localized in the baseepiment.

According to MIKHAILOFF ('11), in Cephalopoda there are four types of the peripheral nerve cells, existing in the muscle and intestinal canal. One of them is said to be the sensory cells located in the muscles, while the others are the associative nerve cells connecting between the peripheral organs and the central nervous system. From the histological character and distribution, they seem also to be corresponding to the intermuscular nerve cells in the earthworm.

Also in Insecta ROGOSINA ('28) found the sensory cells scattered in the muscle and hypodermis. From these facts it may be easily assumed, that the intermuscular nerve cells are widely present in the invertebrata, though they are called very differently according to the kind of animals.

Above all, the intermuscular nerve cells in the earthworm seem to have the most intimate relation with the nervous system of Nemertinea; namely these scattered cells are probably vestiges of the more primitive condition of nerves, as mentioned already by DAWSON, while the relation between the spinal ganglion cells in the vertebrata and the intermuscular nerve cells in the earthworm can not be determined at the present time.

VII. SUMMARY

1. In the ventral nerve cord of *Pheretima communissima* I could discern by the vital staining method with methylene blue various types of motor neurons which were partly unknown to the previous investigators; the axons of these motor cells give off numerous branches on their courses through the neuropile and the segmental nerves.

The interganglionic nerve cells were also observed in abundance; some of them are similar in shape to the motor neurons in the ganglion, and send their axons anteriorly or posteriorly into the neuropile of several adjacent ganglia, while the others are small, unipolar nerve cells which issue one short axon each, and are found especially in a great number in the suboesophageal ganglion, and also in the ganglia of the prostate gland regions.

2. The sensory nerve fibers, entering the ventral cord from the periphery, seem to terminate at the definite region of the neuropile, namely in the LEYDIG's " Punktsubstanz".

3. In *Pheretima communissima* four giant fibers are present in the dorsal part of the ventral cord, i. e. a larger median giant fiber, and a smaller median one, which is located ventrally near the former, and besides two lateral giant fibers. They are all connected segmentally with several giant nerve cells, more ventrally placed, and their branches extend to the neuropile of the ventral cord itself, or go out in the segmental nerve trunks. In addition, we see the so-called ventral giant fibers in the ventral part of the ventral nerve cord.

4. In the suboesophageal ganglion, various characteristic motor and interganglionic nerve cells were found, some of them seem to resemble the neurons described by RETZIUS in that of *Nereis*.

5. In the cerebral ganglion the small characteristic ganglion cells, which do not send their axons outside of this ganglion, are abundantly present, forming the network by their axons at the dorsal surface of the neuropile of this ganglion.

The efferent nerve fibers contained in the cerebral nerve trunks are distinguishable into two different sorts, a) the fibers originating from the nerve cells in the suboesophageal ganglion, and b) the fibers coming from the neurons in the cerebral ganglion.

Most of the afferent fibers contained in the cerebral nerve trunks terminate in the cerebral ganglion itself, but others seem to pass through the circumoesophageal connective to the suboesophageal ganglion.

6. The enteric nerve thickening is connected not only with the

cerebral ganglion, but also with the suboesophageal ganglion by a large number of nerve fibers.

7. The intermuscular nerve cells can be distinguished in three types according to shape, localization, size of cells and stainability of their nuclei; a) the first type is localized along the double or single nerve trunks in the interspace between the circular and longitudinal muscle layers, b) the second type is seen in the circumoesophageal connective and the segmental nerve trunks, c) the third type, which resembles the sensory cells, is present in the baseepiment, and seems to contribute to the formation of the so-called subepidermal network together with the processes of the sensory cells in the epidermis.

In the early embryo which is only 0.5 mm long the first type appears already, arranged segmentally.

8. The large or small sense organs are arranged in three transverse rows in each segment, the middle one of the setal line consisting of the most numerous organs. In the typical segment of the 10 weeks old worm, the number of the sense organs amounts to about 300, though this calculation may not be very exact on account of the insufficiency of the methylene blue staining.

9. According to my estimation the number of ganglion cells and efferent nerve fibers change during the growth from the newly born to the full grown worm, being summarized, in the following manner: a) Among the whole nervous system, the enteric and the intermuscular nerves are developed in the earliest stages, already reaching the full number of the nerve cells in the newly born worm. b) In the typical ganglia of the ventral cord, the ganglion cells attain to the full number in the 5–6 weeks old worm, while the efferent nerve fibers in the neuropile and the segmental nerve trunks amount somewhat later to the full number. c) The number of the ganglion cells in the cerebral and suboesophageal ganglia increase continually during the whole growth period up to the full grown stage. d) In the clitellar and prostate gland regions, the number of the ganglion cells and the efferent nerve fibers increase suddenly in the period of sexual maturation.

10. To compare the nerve cells and the cells of the body wall, the increase of the former in number is far smaller than that of the muscle fibers and epidermal cells. On the other hand, the increase of the nerve cells in size is less remarkable than that of the muscle fibers, but it is more remarkable than that of the epidermal cells.

In general, the size of the nucleus in all the cells examined increases

in the least degree.

11. The numerical relation of nerve cells in the ganglion of the ventral cord to efferent nerve fibers in the neuropile and in the segmental nerve trunks, must not be discussed simply from the total number of nerve cells and efferent fibers on account of the presence of the multipolar motor cells and small interganglionic nerve cells having short process, but in the newly born worm stained with methylene blue the number of efferent fibers in the neuropile and in the segmental nerve trunk is almost equal to that of nerve cells contained in the ganglion of the ventral cord.

Finally, I wish to express my hearty thanks to Prof. S. HATAI for his kind guidance during this investigation, and also to the Saito Gratitude Foundation for making this research possible.

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EXPLANATION OF THE PLATES

PLATE XV.

Fig. 1. The motor neurons giving off their branches to the muscles of the setae.

(obj. $\frac{1}{12}$, oc. 3) ×350.

dN = double nerve, m = muscle fibers, ne = nerve endings, Nl = neuropile, se = setae.

Fig. 2. The small commissural ganglion cells (obj. $\frac{1}{12}$, oc. 3) ×350; drawn under the oil immersion.

ne = nerve ending.

PLATE XVI.

The intermuscular nerve cells in the interspace between the circular and longitudinal muscle layers.

A) The intermuscular nerve cells along the single nerve trunks in the cross-section of the newly born worm; ×75.

B) The intermuscular nerve cells along the double nerve trunks in the cross-section of the newly born worm; ×75.

C) The intermuscular nerve cells in the 0.5 mm long embryo, by the side view; ×135.

D) The intermuscular nerve cells in the 0.5 mm long embryo, by the dorsal view; ×52.

cM = circular muscle layer, ep = epidermis, dN = double nerve trunks.

iC = intermuscular nerve cells, lM = longitudinal muscle layer.

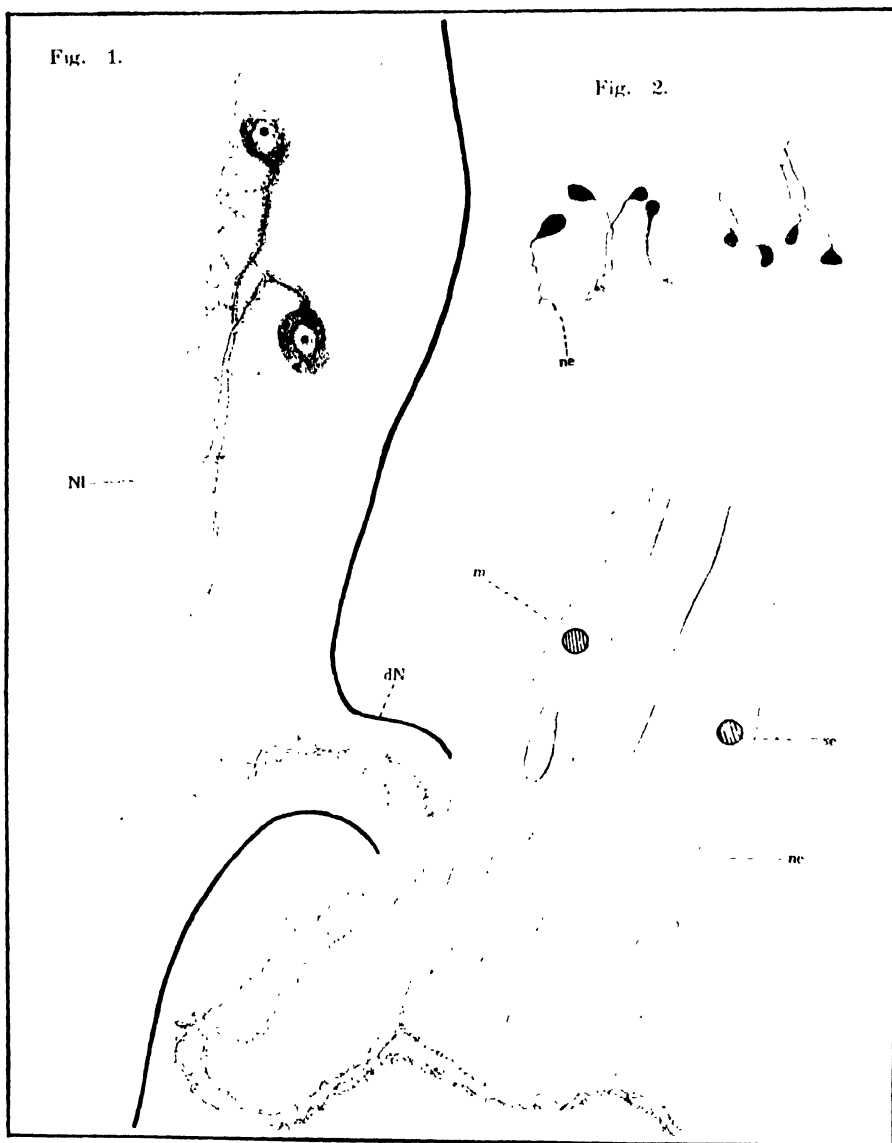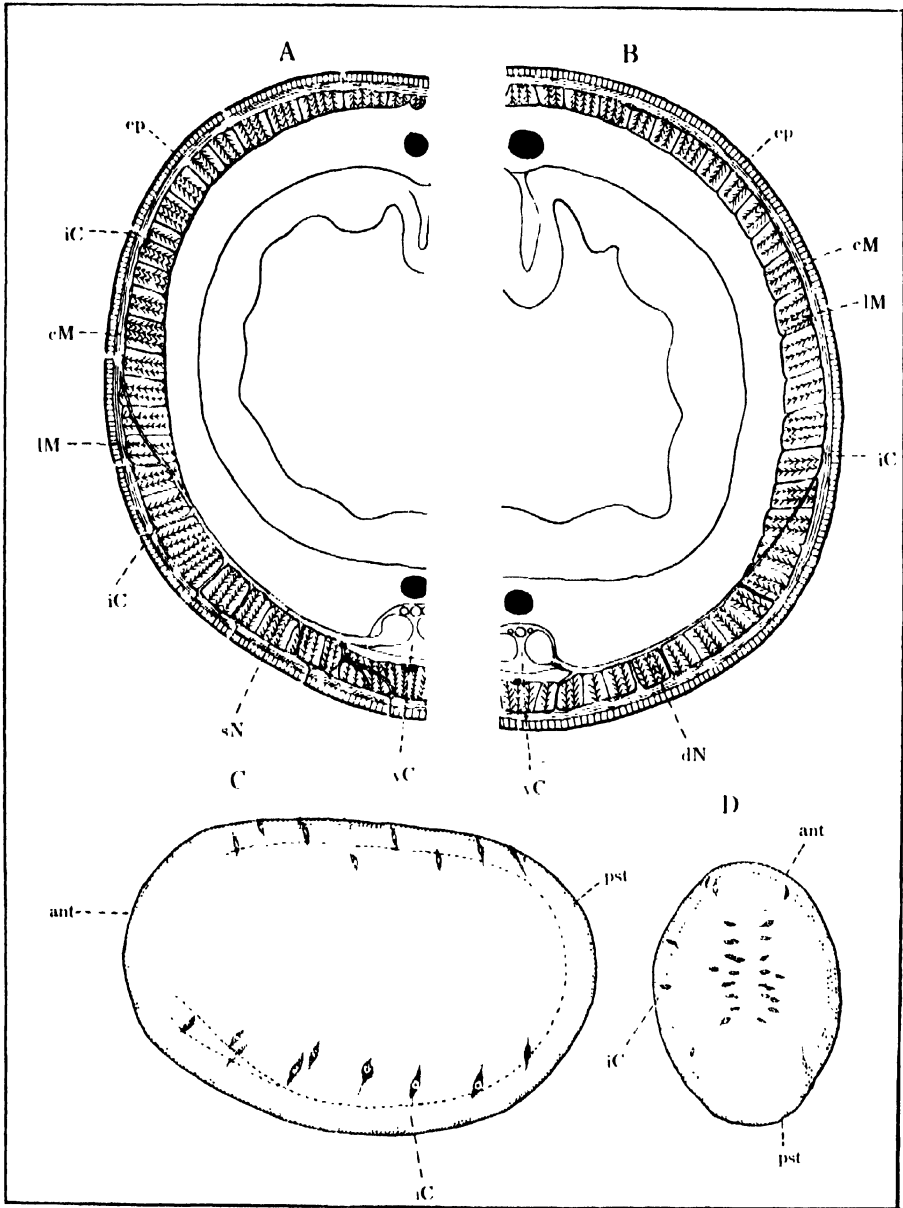sN = single nerve trunks, ant = anterior end, pst = posterior end,

vC = ventral cord.

PLATE XVII.

The characteristic nerve cells in the dorsal cortical layer in the cerebral ganglion of the newly born worm; ×200.
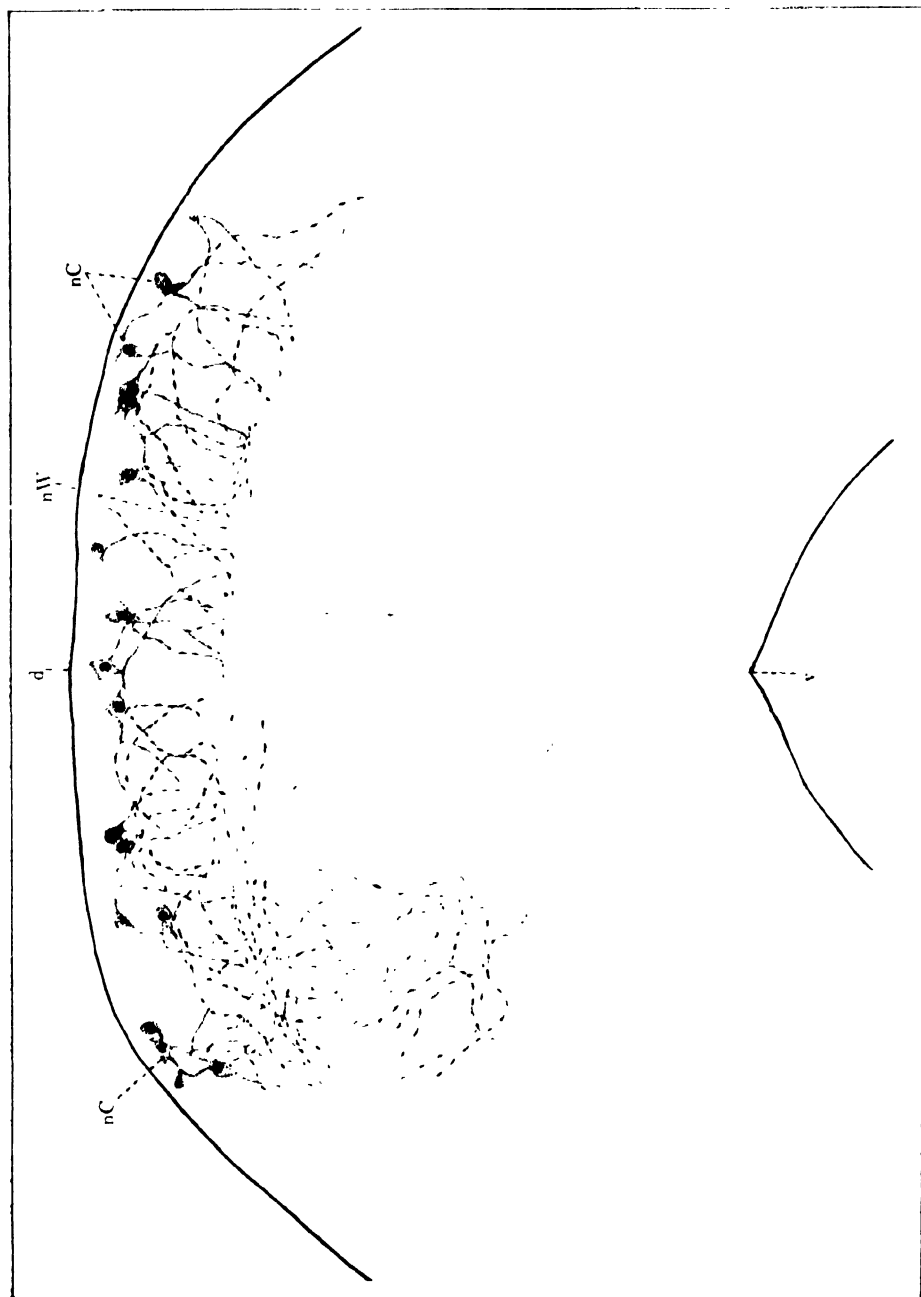
d = dorsal side, nC = characteristic nerve cells,

nW = network portion, v = ventral side.

F. OGAWA: Nervous System of Earthworm.

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